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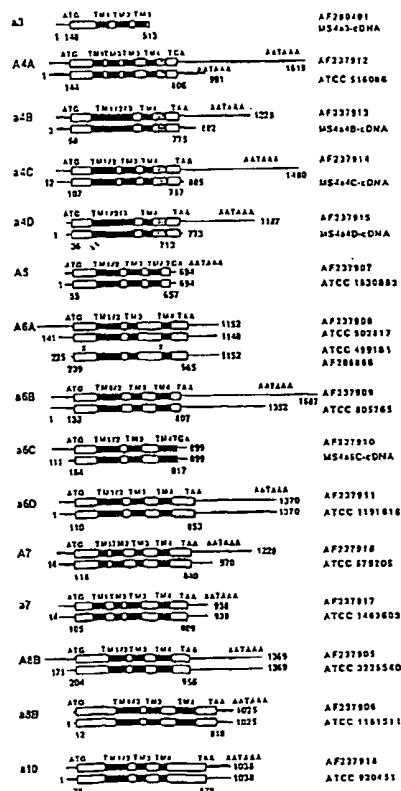
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(54) Title: **IDENTIFICATION OF NOVEL MS4A GENE FAMILY MEMBERS EXPRESSED BY HEMATOPOIETIC CELLS**



(57) Abstract: Isolated nucleic acids encoding MS4A polypeptides, isolated MS4A polypeptides, and uses thereof. The disclosed MS4A nucleic acids and polypeptides can be used to generate a mouse model of atopic disorders, for drug discovery screens, and for therapeutic treatment of atopic disorders or other MS4A-related conditions.

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DescriptionIDENTIFICATION OF NOVEL MS4A GENE FAMILY MEMBERS
EXPRESSED BY HEMATOPOIETIC CELLS

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Cross Reference to Related Applications

This application is based on and claims priority to United States Provisional Application Serial Number 60/254,362, filed December 8, 2000, and United States Provisional Application Serial No. 60/270,057 filed February 20, 2001, herein incorporated by reference in their entirety.

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Grant Statement

This work was supported by NIH grants CA-81776 and CA-54464. Thus, the U.S. Government has rights in the invention.

15

Field of the Invention

The present invention generally relates to a new class of MS4A proteins characterized by a membrane-embedded structure. More particularly, the present invention provides MS4A nucleic acid and polypeptide sequences, chimeric genes comprising disclosed MS4A sequences, antibodies that specifically recognize MS4A polypeptides, and uses thereof.

20

Table of Abbreviations

	ATCC	American Tissue Culture Collection
25	CD20	CD20 B lymphocyte differentiation antigen
	FcεRIβ	high-affinity IgE receptor β chain
	GFP	green fluorescent protein
	htgs	GenBank human genomic database
	HTm4	hematopoietic CD20-like antigen
30	MS4A family	membrane spanning 4-domain family, subfamily A

Background Art

CD20, FcεRIβ, and HTm4 are three cell surface proteins expressed by hematopoietic cells that represent members of a nascent gene family (Adra et al. (1999) *Clin Genet* 55:431-437, Kinet (1999) *Annu Rev Immunol* 17:931-972; Tedder and Engel (1994) *Immunol Today* 15:450-454). The deduced amino acid sequence of human and mouse CD20 first demonstrated a cell surface protein containing four membrane-spanning regions, N- and C-terminal cytoplasmic domains, and an ~50 amino acid loop that serves as the extracellular domain (Einfeld et al. (1988) *EMBO J* 7:711-717; Stamenkovic and Seed (1988) *J Exp Med* 167:1975-1980; Tedder et al. (1988a) *J Immunol* 141:4388-4394; Tedder et al. (1988b) *Proc Natl Acad Sci USA* 85:208-212). Human CD20 shares 20% amino acid sequence identity with FcεRIβ and HTm4 (Adra et al. (1994) *Proc Natl Acad Sci USA* 91:10178-10182, Küster et al. (1992) *J Biol Chem* 267:12782-12787). Moreover, these three proteins have a similar overall structure in man, mouse, and rat with significant sequence identity within the first three membrane-spanning domains (Kinet et al. (1988) *Proc Natl Acad Sci USA* 85:6483-6487; Ra et al. (1989) *Nature* 19:1771-7; Tedder et al., 1988a). In addition, all three genes are located in the same region of human chromosome 11q12-13.1 (Adra et al., 1994; Hupp et al. (1989) *J Immunol* 143:3787-3791; Tedder et al. (1989a) *J Immunol* 142:2555-2559) and mouse chromosome 19 (Hupp et al. 1989; Tedder et al., 1988a). These three genes are therefore likely to have evolved from a common precursor.

Despite structural and sequence conservation between CD20, FcεRIβ and HTm4, transcription of each gene is differentially regulated. CD20 is only expressed by B lymphocytes (Stashenko et al. (1980) *J Immunol* 125:1678-1685; Tedder et al., 1988a). FcεRIβ is expressed by mast cells and basophils (Kinet, 1999). HTm4 is expressed by diverse lymphoid and myeloid origin hematopoietic cells (Adra et al., 1994).

Although the function of HTm4 remains unexplored, CD20 and FcεRIβ have critical roles in cell signaling. CD20 forms a homo- or hetero-tetrameric complex that is functionally important for regulating cell cycle progression

and signal transduction in B lymphocytes (Tedder and Engel, 1994). CD20 additionally regulates transmembrane Ca^{++} conductance, possibly as a functional component of a Ca^{++} -permeable cation channel (Bubien et al. *J Cell Biol* 121:1121-1132; Kanzaki et al. (1997a) *J Biol Chem* 272:14733-14739; Kanzaki et al. (1997b) *J Biol Chem* 272:4964-4969; Kanzaki et al. (1995) *J Biol Chem* 270:13099-13104). FcεRIβ is part of a tetrameric receptor complex consisting of α, β, and two γ chains (Blank et al. (1989) *Nature* 337:187-189). FcεRIβ mediates interactions with IgE-bound antigens that lead to cellular responses such as the degranulation of mast cells. Specifically, the FcεRIβ subunit functions as an amplifier of FcεRIβ-mediated activation signals (Dombrowicz et al. (1998) *Immunity* 8:517-529; Lin et al. (1996) *Cell* 85:985-995). Because of their unique structure and sequence homology, CD20, FcεRIβ, and HTm4 are likely to share overlapping functional properties.

CD20 and FcεRIβ are also important clinically. Antibodies against CD20 are effective in treating non-Hodgkin's lymphoma (McLaughlin et al. (1998) *Oncology* 12:1763-1769; Onrust et al. (1989) *J Biol Chem* 264:15323-15327; Weiner (1999) *Semin Oncol* 26:43-51). Genetic variations at chromosome 11q12-13 can also play a role in the pathogenesis of allergic diseases (Adra et al., 1999; Kinet, 1999). Recent studies suggest that FcεRIβ contributes to such diseases, and other genetic elements in this region likely also contribute to allergic disease.

Since CD20, FcεRIβ, and HTm4 are likely to have evolved by duplication of an ancestral gene, other related proteins might exist that form additional receptor complexes. In view of the clinical importance noted above, the identification of such proteins thus represents a long-felt and ongoing need in the art. To address this need, applicants have identified novel human and mouse proteins that span the cell membrane at least four times and share high levels of amino acid sequence identity with CD20, FcεRIβ, and HTm4. This finding reveals a new gene family that has been designated herein as the MS4A family (membrane spanning 4-domain family, subfamily A). Currently this family contains at least 10 subgroups

(MS4A1 through MS4A12) that encode at least 21 previously unidentified human and mouse proteins expressed by hematopoietic cells and by diverse cell types in non-hematopoietic tissues.

5 Summary of the Invention

The present invention discloses isolated MS4A polypeptides and isolated nucleic acid molecules encoding the same. Preferably, an isolated MS4A polypeptide, or functional portion thereof, comprises a polypeptide encoded by the nucleic acid molecule of any one of the odd numbered SEQ ID NOs:1-37 a polypeptide encoded by a nucleic acid molecule that is substantially identical to any one of the odd-numbered SEQ ID NOs:1-37, a polypeptide fragment encoded by a 20 nucleotide sequence that is identical to a contiguous 20 nucleotide sequence of any one of the odd-numbered SEQ ID NOs:1-37, a polypeptide having an amino acid sequence of any one of the even-numbered SEQ ID NOs:2-38, a polypeptide that is a biological equivalent of any one of the even-numbered SEQ ID NOs:2-38, or a polypeptide that is immunologically cross-reactive with an antibody that shows specific binding with a polypeptide comprising some or all amino acids of any one of the even-numbered SEQ ID NOs:2-38.

20 The present invention further teaches chimeric genes having a heterologous promoter that drives expression of a nucleic acid sequence encoding a MS4A polypeptide. Preferably, the chimeric gene is carried in a vector and introduced into a host cell so that a MS4A polypeptide of the present invention is produced. Preferred host cells include but are not
25 limited to a bacterial cell, a hamster cell, a mouse cell, or a human cell.

In another aspect of the invention, a method is provided for detecting a nucleic acid molecule that encodes a MS4A polypeptide. According to the method, a biological sample having nucleic acid material is hybridized under stringent hybridization conditions to a MS4A nucleic acid molecule of the present invention. Such hybridization enables a nucleic acid molecule of the biological sample and the MS4A nucleic acid molecule to form a detectable duplex structure. Preferably, the MS4A nucleic acid molecule includes some

or all nucleotides of any one of the odd-numbered SEQ ID NOs:1-37. Also preferably, the biological sample comprises human nucleic acid material.

The present invention further teaches an antibody that specifically recognizes a MS4A polypeptide. Preferably, the antibody recognizes some
5 or all amino acids of any one of the even-numbered SEQ ID NOs:2-38. A method for producing a MS4A antibody is also disclosed, and the method comprises recombinantly or synthetically producing a MS4A polypeptide, or portion thereof; formulating the MS4A polypeptide so that it is an effective immunogen; immunizing an animal with the formulated polypeptide to
10 generate an immune response that includes production of MS4A antibodies; and collecting blood serum from the immunized animal containing antibodies that specifically recognize a MS4A polypeptide. Antibody-producing cells can be optionally fused with an immortal cell line whereby a monoclonal antibody that specifically recognizes a MS4A polypeptide can be selected.
15 Preferably, the MS4A polypeptide used as an immunogen includes some or all amino acid sequences of any one the even-numbered SEQ ID NOs:2-38.

A method is also provided for detecting a level of MS4A polypeptide using an antibody that specifically recognizes a MS4A polypeptide. According to the method, a biological sample is obtained from an
20 experimental subject and a control subject, and a MS4A polypeptide is detected in the sample by immunochemical reaction with the MS4A antibody. Preferably, the antibody recognizes amino acids of any one of the even-numbered SEQ ID NOs:2-38, and is prepared according to a method of the present invention for producing such an antibody.

25 The present invention further discloses a method for identifying a compound that modulates MS4A function. The method comprises: exposing an isolated MS4A polypeptide to one or more compounds, and assaying binding of a compound to the isolated MS4A polypeptide. A compound is selected that demonstrates specific binding to the isolated MS4A
30 polypeptide. Preferably, the MS4A polypeptide used in the binding assay of the method includes some or all amino acids of any one of the even-numbered SEQ ID NOs:2-38.

Also provided is a method for identifying a regulator of MS4A gene expression. The method comprises (a) exposing a cell sample with a candidate compound to be tested, the cell sample containing at least one cell containing a DNA construct comprising a modulatable transcriptional regulatory sequence of a MS4A-encoding nucleic acid and a reporter gene which is capable of producing a detectable signal; (b) evaluating an amount of signal produced in relation to a control sample; and (c) identifying a candidate compound as a modulator of MS4A gene expression based on the amount of signal produced in relation to a control sample. Preferably, the modulatable transcriptional regulatory sequence of a MS4A-encoding nucleic acid comprises a sequence that is immediately upstream of the initial coding region of a MS4A gene as set forth in any one of SEQ ID NOs:73-81.

The present invention further provides a method for modulating MS4A function in a subject. According to the method, a pharmaceutical composition is prepared that includes a substance capable of modulating MS4A expression or function, and a carrier. An effective dose of the pharmaceutical composition is administered to a subject, whereby MS4A activity is altered in the subject. Provided are therapeutic methods wherein a change in MS4A activity comprises a shift in the abundance of cell subpopulations expressing said protein, modulation of $[Ca^{2+}]_i$ levels, or altered cell function. In a preferred embodiment, the substance used to perform this method shows specific binding to some or all amino acids of any one of the even-numbered SEQ ID NOs:2-38, and was discovered by a method of the present invention. In another embodiment, MS4A function is disrupted by immunizing a subject with an effective dose of the disclosed MS4A polypeptide. The immune system of the subject produces an antibody that specifically recognizes the MS4A polypeptide, and preferably recognizes some or all of amino acids of any one of the even-numbered SEQ ID NOs:2-38. In a further embodiment, a gene therapy vector is used, the vector comprising a nucleotide sequence encoding a MS4A polypeptide. Alternatively, the gene therapy vector comprises a nucleotide sequence encoding a nucleic acid molecule, a peptide, or a protein that interacts with a

MS4A nucleic acid or polypeptide. Preferably, the subject is a human subject.

Accordingly, it is an object of the present invention to provide novel MS4A nucleic acid and polypeptide sequences, and novel methods relating thereto. This object is achieved in whole or in part by the present invention.

An object of the invention having been stated above, other objects and advantages of the present invention will become apparent to those skilled in the art after a study of the following description of the invention, Figures and non-limiting Examples.

10

Brief Description of the Drawings

Figure 1 depicts cDNAs encoded by fifteen new human or mouse MS4A gene products. Consensus sequences from cDNAs and overlapping ESTs are indicated by their GenBank Accession numbers. Representative full-length cDNAs for each gene product are shown, except for *MS4a3* which was not full-length. 5' and 3' untranslated sequences are shown as horizontal lines with relative nucleotide lengths shown. Coding regions are shown as boxes with translation initiation and termination codons and their relative nucleotide locations shown. Poly(A) attachment signal sequences (AATAAA) are indicated when known. Deduced hydrophobic regions are shown as filled boxes with the predicted membrane-spanning domains shown as TM1-TM4. Additional hydrophobic regions in MS4A4 proteins are shown as shaded boxes. Sites of putative nucleotide polymorphisms in *MS4A6A* are indicated by two (X)s.

Figure 2 depicts exon-intron organization of the human MS4A genes. The maps were constructed by aligning known and predicted MS4A cDNA sequences with human genomic sequences as described in Materials and Methods. Exons are shown as boxes with the predicted translation initiation codons (ATG), transmembrane domains (TM) and termination codons indicated on the top. All exon and intron distances are shown to scale. Gaps indicate where intron distances have not been determined for *MS4A3*, *MS4A4A*, and *MS4A12*. Two long introns present in *MS4A4E* are not to

30

scale but the intron lengths are indicated. Exon numbering for *MS4A1*, and *MS4A2* is as published (Küster et al., 1992; Tedder et al., 1988a; Tedder et al., 1988b).

Figure 3 shows human *MS4A4E* protein and transcript sequences predicted from genomic DNA sequences. *MS4A4E* sequences are compared with human *MS4A4A* cDNA (disclosed herein) and genomic sequences. Gaps were introduced to provide optimal alignment. The boxed AAC sequence near the 5' end of the *MS4A4A* sequence indicates the length of the most 5' *MS4A4A* cDNA sequence. Sequences upstream of this are based on contiguous genomic DNA sequences. Nucleotide numbering is based on the *MS4A4A* cDNA sequence, disclosed herein. Predicted translation initiation codons are shaded. Predicted membrane-spanning regions are underlined. An asterisk indicates predicted translation termination codons. Potential poly-A attachment signal sequences (AATAAA) are boxed.

Figure 4 shows human *MS4A6E* protein and transcript sequences predicted from genomic DNA and overlapping cDNA sequences. Predicted *MS4A6E* transcript sequences are compared with human *MS4A6A* cDNA sequence (disclosed herein). Gaps were introduced in the nucleotide sequence to provide optimal alignment. The 5' end of both transcripts start at 3' splice-acceptor sites which demark the first translated exons for both genes. The 5' end of the putative *MS4A6E* transcript is based on genomic DNA sequence, while the predicted sequences starting at nucleotide 60 were based on both genomic DNA sequences and overlapping cDNA sequences. A gap in the *MS4A6A* sequence is indicated where TM1/2 and TM2 exons are not found in *MS4A6E* transcripts. *MS4A6A* nucleotide numbering is based on the cDNA sequence (disclosed herein). Predicted translation initiation codons are shaded. Predicted membrane-spanning regions are underlined. An asterisk indicates the predicted translation termination codon for the *MS4A6E* protein.

Figure 5 shows human *MS4A10* protein and transcript sequences predicted from human genomic DNA sequences. *MS4A10* nucleotide

sequence is compared with mouse *MS4a10* cDNA sequence (disclosed herein). The 5' end of both transcripts start at 3' splice-acceptor sites which demark the first translated exons for both genes. *MS4a10* nucleotide numbering is based on the cDNA sequence (disclosed herein). Predicted translation initiation codons are shaded. Predicted membrane-spanning regions are underlined. An asterisk indicates predicted translation termination codon for the MS4A10 protein. Potential poly-A attachment signal sequences (AATAAA) are boxed.

Figure 6 depicts a physical linkage map for the MS4A genes. A scheme for chromosome 11 structure is shown on the left with the mapped locations for *MS4A1*, *MS4A2* and *MS4A3* indicated. Representative human BAC clones are shown as vertical black bars with clone names shown on the top and clone size shown at the bottom. All distances are shown to the indicated scale. The distance between and spatial relationship of RP11-312N17 to the four other overlapping BACs shown at the bottom are unknown. Thin bars indicate continuous characterized (mapped or sequenced) regions of DNA that contain identified MS4A genes. When the relative position of this region of DNA is known relative to the representative BACs that are shown, the thin bars overlay the BAC. The mapped position of each MS4A gene is indicated on the right with the relative direction of gene translation indicated by arrows (→). In some cases, approximate distances between MS4A genes (termination codons to the translation initiation codon for the next gene) are indicated in base pairs (bp). In some cases, approximate MS4A gene size is indicated showing the distance between predicted translation initiation codons and translation termination codons as show in Figure 7.

Figure 7 depicts deduced amino acid sequences for CD20 (human A1, SEQ ID NO:40; mouse a1, SEQ ID NO:48), FcεRIβ (human A2, SEQ ID NO:42; mouse a2, SEQ ID NO:50), HTm4 (human A3, SEQ ID NO:44; mouse a3, SEQ ID NO:20), and 19 new MS4A (human) (even-numbered SEQ ID NOs:2-18, 46) and MS4a (mouse and pig) proteins (even-numbered SEQ ID NOs:22-38, 56). Gaps were introduced to optimize alignments.

Numbers represent predicted residue positions. The predicted membrane-spanning regions (TM1-TM4) are indicated. Predicted intron|exon splice junctions are indicated by vertical bars where information was available. Amino acids common to 10 or more proteins are shaded. *indicates partial sequence for the MS4a3 protein. CD20, FcεRIβ, and HTm4 sequences and known intron|exon borders (SEQ ID NOs:39-44, 47-50) are as published (Adra et al., 1994; Küster et al., 1992; Ra et al., 1989; Tedder et al., 1988a; Tedder et al., 1989b; Tedder et al., 1988b). MS4A12 represents a conceptual translation (SEQ ID NO:46) of a human colon mucosa cDNA sequence (GenBank AK000224, SEQ ID NO:45), and MS4a12 represents a conceptual translation (SEQ ID NO:56) of a homologous cDNA sequence from pig (GenBank AJ236932, SEQ ID NO:55).

Figure 8 depicts UPGMA (unweighted pair group method using arithmetic averages) tree of deduced MS4A and MS4a protein sequences. Horizontal tree branch length is a measure of sequence relatedness. For example, MS4a4B and MS4a4C are the most similar in sequence, while CD20 (MS4A1) sequences were the most divergent from other family members. The MS4a12p sequence was from pig, while all other MS4a sequences were from mouse. The UPGMA tree was generated using Geneworks version 2.0 (IntelliGenetics, Inc., Mountain View, California, USA).

Figure 9 shows immunofluorescent detection of CD20 expression during B cell development. Single cell suspensions of leukocytes were isolated from wild-type mice, stained using MB20-13 (visualized using a PE-conjugated, anti-mouse IgG3 antibody) and anti-B220 (FITC-conjugated) monoclonal antibodies, and examined by two-color immunofluorescence staining with flow cytometry analysis. Quadrant gates indicate negative and positive populations of cells as determined using isotype-matched control monoclonal antibodies. The gated cell populations correspond to the cells described in Table 7, and are shown for reference. These results are representative of those obtained with six (6) two month-old wild type mice.

Figure 10 summarizes the strategy for targeted disruption of the mouse *CD20* gene.

Figure 10A shows genomic clones encoding *CD20*.

Figure 10B shows the intron-exon organization of the wild type *CD20* allele containing exons 5-8 (shaded squares).

Figure 10C shows the structure of the *CD20* targeting vector.

Figure 10D shows the predicted structure of the *CD20* allele after gene targeting in ES cells by homologous recombination. The *EcoR* V restriction site in exon 6 is deleted as indicated.

Figure 10E presents Southern blot analysis of tail DNA from two wild type and four *CD20*^{-/-} mice. Genomic DNA was digested with *EcoR* V, transferred to nitrocellulose and hybridized with the 5' probe indicated in (D).

Figure 10F shows PCR amplification of genomic DNA from wild type and *CD20*^{-/-} mice using primers that bind in exons 6 and 7. Amplification of glyceraldehyde-3-phosphate dehydrogenase (G3PDH) is shown as a positive control.

Figure 10G shows PCR amplification of cDNA generated from splenic RNA of wild type and *CD20*^{-/-} mice. Each reaction mixture contained a sense primer that hybridized with sequences encoded by exon 3 and antisense primers that hybridized with either exon 6 or *Neof* gene promoter sequences.

Figures 10H and 10I show reactivity of the MB20-13 monoclonal antibody with *CD20* cDNA-transfected (thick line) or untransfected (dashed line) 300.19 cells (Figure 10H) or Chinese Hamster Ovary (CHO) cells (Figure 10I). The thin lines represent *CD20* cDNA-transfected cells stained with secondary antibody alone or an isotype-control monoclonal antibody. Indirect immunofluorescence staining was visualized by flow cytometry analysis.

Figure 10J shows immunofluorescent staining of splenocytes from *CD20*^{-/-} or wild type mice with MB20-13 (visualized using a PE-conjugated, anti-mouse IgG3 antibody) and anti-B220 (FITC-conjugated) monoclonal antibodies. Splenocytes from *CD20*^{-/-} mice generated histograms identical to

those obtained without MB20-1 monoclonal antibody present, using the secondary antibody alone.

Figure 11 depicts immunofluorescent detection of B lymphocyte subpopulations in CD20^{-/-} and wild type mice. Lymphocytes were isolated and examined by two color immunofluorescent staining with flow cytometry analysis. Quadrants delineated by squares indicate negative and positive populations of cells as determined using unreactive monoclonal antibody controls. The gated cell populations correspond to the cells described in Table 7 that represent at least 6 mice of each genotype.

Figure 12 shows altered signal transduction in CD20^{-/-} B cells. Figure 12 also shows CD19 expression by splenocytes from CD20^{-/-} (thin line) and wild type (thick line) mice. Immunofluorescence staining using PE-conjugated anti-CD19 monoclonal antibody with flow cytometry analysis. The dashed line represents staining of wild type splenocytes with a control antibody.

Figure 12A presents calcium responses induced by BCR or CD19 ligation in CD20^{-/-} and wild type B cells. Splenocytes were loaded with 1 μ M indo-1-AM ester and B cells were stained with FITC-conjugated anti-B220 antibody. At 1 min (arrow), optimal concentrations of goat anti-IgM F(ab')₂ antibody fragments, anti-CD19 monoclonal antibody or Thapsigargin were added, with or without EGTA present. Increased ratios of indo-1 fluorescence indicate increased [Ca²⁺]_i. Results represent those from at least four experiments.

Figure 12B presents assays of tyrosine phosphorylation of proteins from purified splenic B cells of CD20^{-/-} and wild type mice. B cells (2 x 10⁷/sample) were incubated with anti-IgM antibody for the times shown and detergent lysed. Proteins were resolved by SDS-PAGE, transferred to nitrocellulose and immunoblotted with anti-phosphotyrosine (anti-PTyr) antibody. The blot was stripped and reprobed with anti-SHP-1 antibody as a control for equivalent protein loading. Western blots from two of three experiments are shown to demonstrate the range of results.

Detailed Description of the Invention

The present invention provides isolated nucleic acids encoding MS4A polypeptides (representative embodiments set forth as the odd-numbered SEQ ID NOs:1-37), isolated MS4A polypeptides (representative
5 embodiments set forth as the even-numbered SEQ ID NOs:2-38), and uses thereof. The disclosed MS4A nucleic acids and polypeptides can be used according to methods of the present invention for drug discovery screens, for therapeutic treatment of atopic conditions, and for therapeutic regulation of $[Ca^{2+}]_i$ levels, among other uses.

10

I. DEFINITIONS

While the following terms are believed to be well understood by one of ordinary skill in the art, the following definitions are set forth to facilitate explanation of the invention. The entire contents of all publications
15 mentioned herein, including the discussion of the background art presented above, are hereby fully incorporated by reference.

I.A. MS4A nucleic acids

The nucleic acid molecules provided by the present invention include the isolated nucleic acid molecules of any one of the odd-numbered SEQ ID
20 NOs:1-37, sequences substantially similar to sequences of any one of the odd-numbered SEQ ID NOs:1-37, conservative variants thereof, subsequences and elongated sequences thereof, complementary DNA molecules, and corresponding RNA molecules. The present invention also encompasses genes, cDNAs, chimeric genes, and vectors comprising
25 disclosed MS4A nucleic acid sequences.

The term "nucleic acid molecule" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides which have similar
30 properties as the reference natural nucleic acid. Unless otherwise indicated, a particular nucleotide sequence also implicitly encompasses conservatively modified variants thereof (e.g. degenerate codon substitutions),

complementary sequences, subsequences, elongated sequences, as well as the sequence explicitly indicated. The terms "nucleic acid molecule" or "nucleotide sequence" can also be used in place of "gene", "cDNA", or "mRNA". Nucleic acids can be derived from any source, including any
5 organism.

The term "isolated", as used in the context of a nucleic acid molecule, indicates that the nucleic acid molecule exists apart from its native environment and is not a product of nature. An isolated DNA molecule can exist in a purified form or can exist in a non-native environment such as a
10 transgenic host cell.

The term "purified", when applied to a nucleic acid, denotes that the nucleic acid is essentially free of other cellular components with which it is associated in the natural state. Preferably, a purified nucleic acid molecule is a homogeneous dry or aqueous solution. The term "purified" denotes that
15 a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. Particularly, it means that the nucleic acid is at least about 50% pure, more preferably at least about 85% pure, and most preferably at least about 99% pure.

The term "substantially identical", in the context of two nucleotide or
20 amino acid sequences, can also be defined as two or more sequences or subsequences that have at least 60%, preferably 80%, more preferably 90-95%, and most preferably at least 99% nucleotide or amino acid sequence identity, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms
25 (described herein below under the heading Nucleotide and Amino Acid Sequence Comparisons) or by visual inspection. Preferably, the substantial identity exists in nucleotide sequences of at least 50 residues, more preferably in nucleotide sequence of at least about 100 residues, more preferably in nucleotide sequences of at least about 150 residues, and most
30 preferably in nucleotide sequences comprising complete coding sequences. In one aspect, polymorphic sequences can be substantially identical sequences. The term "polymorphic" refers to the occurrence of two or more

genetically determined alternative sequences or alleles in a population. An allelic difference can be as small as one base pair.

Another indication that two nucleotide sequences are substantially identical is that the two molecules specifically or substantially hybridize to each other under stringent conditions. In the context of nucleic acid hybridization, two nucleic acid sequences being compared can be designated a "probe" and a "target". A "probe" is a reference nucleic acid molecule, and a "target" is a test nucleic acid molecule, often found within a heterogeneous population of nucleic acid molecules. A "target sequence" is synonymous with a "test sequence".

A preferred nucleotide sequence employed for hybridization studies or assays includes probe sequences that are complementary to or mimic at least an about 14 to 40 nucleotide sequence of a nucleic acid molecule of the present invention. Preferably, probes comprise 14 to 20 nucleotides, or even longer where desired, such as 30, 40, 50, 60, 100, 200, 300, or 500 nucleotides or up to the full length of any of those set forth as the odd-numbered SEQ ID NOs:1-37. Such fragments can be readily prepared by, for example, directly synthesizing the fragment by chemical synthesis, by application of nucleic acid amplification technology, or by introducing selected sequences into recombinant vectors for recombinant production.

The phrase "hybridizing specifically to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent conditions when that sequence is present in a complex nucleic acid mixture (e.g., total cellular DNA or RNA). The phrase "binds substantially to" refers to complementary hybridization between a probe nucleic acid molecule and a target nucleic acid molecule and embraces minor mismatches that can be accommodated by reducing the stringency of the hybridization media to achieve the desired hybridization.

"Stringent hybridization conditions" and "stringent hybridization wash conditions" in the context of nucleic acid hybridization experiments such as Southern and Northern blot analysis are both sequence- and environment-dependent. Longer sequences hybridize specifically at higher temperatures.

An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) Laboratory Techniques in Biochemistry and Molecular Biology-Hybridization with Nucleic Acid Probes, part I chapter 2, Elsevier, New York, New York. Generally, highly stringent hybridization and wash conditions are
5 selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. Typically, under "stringent conditions" a probe will hybridize specifically to its target subsequence, but to no other sequences.

The T_m is the temperature (under defined ionic strength and pH) at
10 which 50% of the target sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the T_m for a particular probe. An example of stringent hybridization conditions for Southern or Northern Blot analysis of complementary nucleic acids having more than about 100 complementary residues is overnight hybridization in 50%
15 formamide with 1 mg of heparin at 42°C. An example of highly stringent wash conditions is 15 minutes in 0.1 5 M NaCl at 65°C. An example of stringent wash conditions is 15 minutes in 0.2X SSC buffer at 65°C (See Sambrook et al. eds. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York for a
20 description of SSC buffer). Often, a high stringency wash is preceded by a low stringency wash to remove background probe signal. An example of medium stringency wash conditions for a duplex of more than about 100 nucleotides, is 15 minutes in 1X SSC at 45°C. An example of low stringency wash for a duplex of more than about 100 nucleotides, is 15 minutes in 4-6X
25 SSC at 40°C. For short probes (e.g., about 10 to 50 nucleotides), stringent conditions typically involve salt concentrations of less than about 1.0 M Na^+ ion, typically about 0.01 to 1.0 M Na^+ ion concentration (or other salts) at pH 7.0-8.3, and the temperature is typically at least about 30°C. Stringent conditions can also be achieved with the addition of destabilizing agents
30 such as formamide. In general, a signal to noise ratio of 2-fold (or higher) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization.

The following are examples of hybridization and wash conditions that can be used to clone homologous nucleotide sequences that are substantially identical to reference nucleotide sequences of the present invention: a probe nucleotide sequence preferably hybridizes to a target
5 nucleotide sequence in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C followed by washing in 2X SSC, 0.1% SDS at 50°C; more preferably, a probe and target sequence hybridize in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C followed by washing in 1X SSC, 0.1% SDS at 50°C; more preferably, a probe and target sequence
10 hybridize in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C followed by washing in 0.5X SSC, 0.1% SDS at 50°C; more preferably, a probe and target sequence hybridize in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C followed by washing in 0.1X SSC, 0.1% SDS at 50°C; more preferably, a probe and target sequence hybridize in 7%
15 sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C followed by washing in 0.1X SSC, 0.1% SDS at 65°C.

A further indication that two nucleic acid sequences are substantially identical is that proteins encoded by the nucleic acids are substantially identical, share an overall three-dimensional structure, are biologically
20 functional equivalents, or are immunologically cross-reactive. These terms are defined further under the heading MS4A Polypeptides herein below. Nucleic acid molecules that do not hybridize to each other under stringent conditions are still substantially identical if the corresponding proteins are substantially identical. This can occur, for example, when two nucleotide
25 sequences are significantly degenerate as permitted by the genetic code.

The term "conservatively substituted variants" refers to nucleic acid sequences having degenerate codon substitutions wherein the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzner et al. (1991) *Nucleic Acids Res* 19:5081;
30 Ohtsuka et al. (1985) *J Biol Chem* 260:2605-2608; Rossolini et al. (1994) *Mol Cell Probes* 8:91-98).

The term "subsequence" refers to a sequence of nucleic acids that comprises a part of a longer nucleic acid sequence. An exemplary subsequence is a probe, described herein above, or a primer. The term "primer" as used herein refers to a contiguous sequence comprising about 8
5 or more deoxyribonucleotides or ribonucleotides, preferably 10-20 nucleotides, and more preferably 20-30 nucleotides of a selected nucleic acid molecule. The primers of the invention encompass oligonucleotides of sufficient length and appropriate sequence so as to provide initiation of polymerization on a nucleic acid molecule of the present invention.

10 The term "elongated sequence" refers to an addition of nucleotides (or other analogous molecules) incorporated into the nucleic acid. For example, a polymerase (e.g., a DNA polymerase), e.g., a polymerase which adds sequences at the 3' terminus of the nucleic acid molecule. In addition, the nucleotide sequence can be combined with other DNA sequences, such
15 as promoters, promoter regions, enhancers, polyadenylation signals, intronic sequences, additional restriction enzyme sites, multiple cloning sites, and other coding segments.

The term "complementary sequence", as used herein, indicates two nucleotide sequences that comprise antiparallel nucleotide sequences
20 capable of pairing with one another upon formation of hydrogen bonds between base pairs. As used herein, the term "complementary sequences" means nucleotide sequences which are substantially complementary, as can be assessed by the same nucleotide comparison set forth above, or is defined as being capable of hybridizing to the nucleic acid segment in
25 question under relatively stringent conditions such as those described herein. A particular example of a complementary nucleic acid segment is an antisense oligonucleotide.

The term "gene" refers broadly to any segment of DNA associated with a biological function. A gene encompasses sequences including but not
30 limited to a coding sequence, a promoter region, a cis-regulatory sequence, a non-expressed DNA segment is a specific recognition sequence for regulatory proteins, a non-expressed DNA segment that contributes to gene

expression, a DNA segment designed to have desired parameters, or combinations thereof. A gene can be obtained by a variety of methods, including cloning from a biological sample, synthesis based on known or predicted sequence information, and recombinant derivation of an existing
5 sequence.

The term "gene expression" generally refers to the cellular processes by which a biologically active polypeptide is produced from a DNA sequence.

The present invention also encompasses chimeric genes comprising
10 the disclosed MS4A sequences. The term "chimeric gene", as used herein, refers to a promoter region operably linked to a MS4A coding sequence, a nucleotide sequence producing an antisense RNA molecule, a RNA molecule having tertiary structure, such as a hairpin structure, or a double-stranded RNA molecule.

15 The term "operably linked", as used herein, refers to a promoter region that is connected to a nucleotide sequence in such a way that the transcription of that nucleotide sequence is controlled and regulated by that promoter region. Techniques for operatively linking a promoter region to a nucleotide sequence are well known in the art.

20 The terms "heterologous gene", "heterologous DNA sequence", "heterologous nucleotide sequence", "exogenous nucleic acid molecule", or "exogenous DNA segment", as used herein, each refer to a sequence that originates from a source foreign to an intended host cell or, if from the same source, is modified from its original form. Thus, a heterologous gene in a
25 host cell includes a gene that is endogenous to the particular host cell but has been modified, for example by mutagenesis or by isolation from native cis-regulatory sequences. The terms also include non-naturally occurring multiple copies of a naturally occurring nucleotide sequence. Thus, the terms refer to a DNA segment that is foreign or heterologous to the cell, or
30 homologous to the cell but in a position within the host cell nucleic acid wherein the element is not ordinarily found.

The term "promoter region" defines a nucleotide sequence within a

gene that is positioned 5' to a coding sequence of a same gene and functions to direct transcription of the coding sequence. The promoter region includes a transcriptional start site and at least one cis-regulatory element. The present invention encompasses nucleic acid sequences that
5 comprise a promoter region of a MS4A gene, or functional portion thereof.

The term "cis-acting regulatory sequence" or "cis-regulatory motif" or "response element", as used herein, each refer to a nucleotide sequence that enables responsiveness to a regulatory transcription factor. Responsiveness can encompass a decrease or an increase in
10 transcriptional output and is mediated by binding of the transcription factor to the DNA molecule comprising the response element.

The term "transcription factor" generally refers to a protein that modulates gene expression by interaction with the cis-regulatory element and cellular components for transcription, including RNA Polymerase, Transcription Associated Factors (TAFs), chromatin-remodeling proteins,
15 and any other relevant protein that impacts gene transcription.

A "functional portion" of a promoter gene fragment is a nucleotide sequence within a promoter region that is required for normal gene transcription. To determine nucleotide sequences that are functional, the expression of a reporter gene is assayed when variably placed under the
20 direction of a promoter region fragment.

Promoter region fragments can be conveniently made by enzymatic digestion of a larger fragment using restriction endonucleases or DNase I. Preferably, a functional promoter region fragment comprises about 5000 nucleotides, more preferably 2000 nucleotides, more preferably about 1000
25 nucleotides. Even more preferably a functional promoter region fragment comprises about 500 nucleotides, even more preferably a functional promoter region fragment comprises about 100 nucleotides, and even more preferably a functional promoter region fragment comprises about 20
30 nucleotides.

The terms "reporter gene" or "marker gene" or "selectable marker" each refer to a heterologous gene encoding a product that is readily

observed and/or quantitated. A reporter gene is heterologous in that it originates from a source foreign to an intended host cell or, if from the same source, is modified from its original form. Non-limiting examples of detectable reporter genes that can be operably linked to a transcriptional regulatory region can be found in Alam & Cook (1990) *Anal Biochem* 188:245-254 and PCT International Publication No. WO 97/47763. Preferred reporter genes for transcriptional analyses include the *lacZ* gene (See, e.g., Rose & Botstein (1983) *Meth Enzymol* 101:167-180), Green Fluorescent Protein (GFP) (Cubitt et al. (1995) *Trends Biochem Sci* 20:448-455), luciferase, or chloramphenicol acetyl transferase (CAT). Preferred reporter genes for methods to produce transgenic animals include but are not limited to antibiotic resistance genes, and more preferably the antibiotic resistance gene confers neomycin resistance. Any suitable reporter and detection method can be used, and it will be appreciated by one of skill in the art that no particular choice is essential to or a limitation of the present invention.

An amount of reporter gene can be assayed by any method for qualitatively or preferably, quantitatively determining presence or activity of the reporter gene product. The amount of reporter gene expression directed by each test promoter region fragment is compared to an amount of reporter gene expression to a control construct comprising the reporter gene in the absence of a promoter region fragment. A promoter region fragment is identified as having promoter activity when there is significant increase in an amount of reporter gene expression in a test construct as compared to a control construct. The term "significant increase", as used herein, refers to an quantified change in a measurable quality that is larger than the margin of error inherent in the measurement technique, preferably an increase by about 2-fold or greater relative to a control measurement, more preferably an increase by about 5-fold or greater, and most preferably an increase by about 10-fold or greater.

The present invention further includes vectors comprising the disclosed MS4A sequences, including plasmids, cosmids, and viral vectors.

The term "vector", as used herein refers to a DNA molecule having sequences that enable its replication in a compatible host cell. A vector also includes nucleotide sequences to permit ligation of nucleotide sequences within the vector, wherein such nucleotide sequences are also replicated in a compatible host cell. A vector can also mediate recombinant production of a MS4A polypeptide, as described further herein below. Preferred vectors include but are not limited to pBluescript (Stratagene), pUC18, pBLCAT3 (Luckow & Schutz (1987) *Nucleic Acids Res* 15:5490), pLNTK (Gorman et al. (1996) *Immunity* 5:241-252), and pBAD/gIII (Stratagene). A preferred host cell is a mammalian cell; more preferably the cell is a Chinese hamster ovary cell, a HeLa cell, a baby hamster kidney cell, or a mouse cell; even more preferably the cell is a human cell.

Nucleic acids of the present invention can be cloned, synthesized, recombinantly altered, mutagenized, or combinations thereof. Standard recombinant DNA and molecular cloning techniques used to isolate nucleic acids are well known in the art. Exemplary, non-limiting methods are described by Sambrook et al., eds. (1989); by Silhavy et al. (1984) Experiments with Gene Fusions, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; by Ausubel et al. (1992) Current Protocols in Molecular Biology, John Wiley and Sons, Inc., New York, New York; and by Glover, ed. (1985) DNA Cloning: A Practical Approach, MRL Press, Ltd., Oxford, United Kingdom. Site-specific mutagenesis to create base pair changes, deletions, or small insertions are also well known in the art as exemplified by publications, see, e.g., Adelman et al., (1983) *DNA* 2:183; Sambrook et al. (1989).

Sequences detected by methods of the invention can be detected, subcloned, sequenced, and further evaluated by any measure well known in the art using any method usually applied to the detection of a specific DNA sequence including but not limited to dideoxy sequencing, PCR, oligomer restriction (Saiki et al. (1985) *Bio/Technology* 3:1008-1012), allele-specific oligonucleotide (ASO) probe analysis (Conner et al. (1983) *Proc Natl Acad Sci USA* 80:278), and oligonucleotide ligation assays (OLAs) (Landgren et.

al. (1988) *Science* 241:1007). Molecular techniques for DNA analysis have been reviewed (Landgren et. al. (1988) *Science* 242:229-237).

I.B. MS4A Polypeptides

The polypeptides provided by the present invention include the
5 isolated polypeptides set forth as the even-numbered SEQ ID NOs:2-38, polypeptides substantially similar to the even-numbered SEQ ID NOs:2-38, MS4A polypeptide fragments, fusion proteins comprising MS4A amino acid sequences, biologically functional analogs, and polypeptides that cross-react with an antibody that specifically recognizes a MS4A polypeptide.

10 The term "isolated", as used in the context of a polypeptide, indicates that the polypeptide exists apart from its native environment and is not a product of nature. An isolated polypeptide can exist in a purified form or can exist in a non-native environment such as, for example, in a transgenic host cell.

15 The term "purified", when applied to a polypeptide, denotes that the polypeptide is essentially free of other cellular components with which it is associated in the natural state. Preferably, a polypeptide is a homogeneous solid or aqueous solution. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel
20 electrophoresis or high performance liquid chromatography. A polypeptide which is the predominant species present in a preparation is substantially purified. The term "purified" denotes that a polypeptide gives rise to essentially one band in an electrophoretic gel. Particularly, it means that the polypeptide is at least about 50% pure, more preferably at least about 85%
25 pure, and most preferably at least about 99% pure.

The term "substantially identical" in the context of two or more polypeptides sequences is measured by (a) polypeptide sequences having about 35%, or 45%, or preferably from 45-55%, or more preferably 55-65%, or most preferably 65% or greater amino acids which are identical or
30 functionally equivalent. Percent "identity" and methods for determining identity are defined herein below under the heading Nucleotide and Amino Acid Sequence Comparisons.

Substantially identical polypeptides also encompass two or more polypeptides sharing a conserved three-dimensional structure. Computational methods can be used to compare structural representations, and structural models can be generated and easily tuned to identify similarities around important active sites or ligand binding sites. See Henikoff et al. (2000) *Electrophoresis* 21(9):1700-1706; Huang et al. (2000) *Pac Symp Biocomput* 230-241; Saqi et al. (1999) *Bioinformatics* 15(6):521-522; and Barton (1998) *Acta Crystallogr D Biol Crystallogr* 54:1139-1146.

The term "functionally equivalent" in the context of amino acid sequences is well known in the art and is based on the relative similarity of the amino acid side-chain substituents. See Henikoff & Henikoff (2000) *Adv Protein Chem* 54:73-97. Relevant factors for consideration include side-chain hydrophobicity, hydrophilicity, charge, and size. For example, arginine, lysine, and histidine are all positively charged residues; that alanine, glycine, and serine are all of similar size; and that phenylalanine, tryptophan, and tyrosine all have a generally similar shape. By this analysis, described further herein below, arginine, lysine, and histidine; alanine, glycine, and serine; and phenylalanine, tryptophan, and tyrosine; are defined herein as biologically functional equivalents.

In making biologically functional equivalent amino acid substitutions, the hydropathic index of amino acids can be considered. Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics, these are: isoleucine (+ 4.5); valine (+ 4.2); leucine (+ 3.8); phenylalanine (+ 2.8); cysteine (+ 2.5); methionine (+ 1.9); alanine (+ 1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is generally understood in the art (Kyte et al. (1982) *J Mol Biol* 157:105.). It is known that certain amino acids can be substituted for other amino acids having a similar hydropathic index

or score and still retain a similar biological activity. In making changes based upon the hydropathic index, the substitution of amino acids whose hydropathic indices are within ± 2 of the original value is preferred, those which are within ± 1 of the original value are particularly preferred, and those within ± 0.5 of the original value are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Patent No. 4,554,101 states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, *i.e.* with a biological property of the protein. It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent protein.

As detailed in U.S. Patent No. 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+ 3.0); lysine (+ 3.0); aspartate (+ 3.0 \pm 1); glutamate (+ 3.0 \pm 1); serine (+ 0.3); asparagine (+ 0.2); glutamine (+ 0.2); glycine (0); threonine (-0.4); proline (-0.5 \pm 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4).

In making changes based upon similar hydrophilicity values, the substitution of amino acids whose hydrophilicity values are within ± 2 of the original value is preferred, those which are within ± 1 of the original value are particularly preferred, and those within ± 0.5 of the original value are even more particularly preferred.

The present invention also encompasses MS4A polypeptide fragments or functional portions of a MS4A polypeptide. Such functional portion need not comprise all or substantially all of the amino acid sequence of a native MS4A gene product. The term "functional" includes any biological activity or feature of MS4A, including immunogenicity.

The present invention also includes longer sequences of a MS4A polypeptide, or portion thereof. For example, one or more amino acids can

be added to the N-terminus or C-terminus of a MS4A polypeptide. Fusion proteins comprising MS4A polypeptide sequences are also provided within the scope of the present invention. Methods of preparing such proteins are known in the art.

5 The present invention also encompasses functional analogs of a MS4A polypeptide. Functional analogs share at least one biological function with a MS4A polypeptide. An exemplary function is immunogenicity. In the context of amino acid sequence, biologically functional analogs, as used
10 can be substituted. Functional analogs can be created at the level of the corresponding nucleic acid molecule, altering such sequence to encode desired amino acid changes. In one embodiment, changes can be introduced to improve the antigenicity of the protein. In another embodiment, a MS4A polypeptide sequence is varied so as to assess the
15 activity of a mutant MS4A polypeptide.

 The present invention also encompasses recombinant production of the disclosed MS4A polypeptides. Briefly, a nucleic acid sequence encoding a MS4A polypeptide, or portion thereof, is cloned into a expression cassette, the cassette is introduced into a host organism, where it is recombinantly
20 produced.

 The term "expression cassette" as used herein means a DNA sequence capable of directing expression of a particular nucleotide sequence in an appropriate host cell, comprising a promoter operably linked to the nucleotide sequence of interest which is operably linked to termination
25 signals. It also typically comprises sequences required for proper translation of the nucleotide sequence. The expression cassette comprising the nucleotide sequence of interest can be chimeric. The expression cassette can also be one which is naturally occurring but has been obtained in a recombinant form useful for heterologous expression.

30 The expression of the nucleotide sequence in the expression cassette can be under the control of a constitutive promoter or an inducible promoter which initiates transcription only when the host cell is exposed to some

particular external stimulus. Exemplary promoters include Simian virus 40 early promoter, a long terminal repeat promoter from retrovirus, an action promoter, a heat shock promoter, and a metallothien protein. In the case of a multicellular organism, the promoter and promoter region can direct
5 expression to a particular tissue or organ or stage of development. Exemplary tissue-specific promoter regions include a MS4A promoter, described herein. Suitable expression vectors which can be used include, but are not limited to, the following vectors or their derivatives: human or animal viruses such as vaccinia virus or adenovirus, yeast vectors,
10 bacteriophage vectors (e.g., lambda phage), and plasmid and cosmids DNA vectors.

The term "host cell", as used herein, refers to a cell into which a heterologous nucleic acid molecule has been introduced. Transformed cells, tissues, or organisms are understood to encompass not only the end product
15 of a transformation process, but also transgenic progeny thereof.

A host cell strain can be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. For example, different host cells have characteristic and specific mechanisms for the translational and post-
20 transactional processing and modification (e.g., glycosylation, phosphorylation of proteins). Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. Expression in a bacterial system can be used to produce a non-glycosylated core protein product. Expression in yeast will produce a
25 glycosylated product. Expression in animal cells can be used to ensure "native" glycosylation of a heterologous protein.

Expression constructs are transfected into a host cell by any standard method, including electroporation, calcium phosphate precipitation, DEAE-Dextran transfection, liposome-mediated transfection, and infection using a
30 retrovirus. The MS4A-encoding nucleotide sequence carried in the expression construct can be stably integrated into the genome of the host or it can be present as an extrachromosomal molecule.

Isolated polypeptides and recombinantly produced polypeptides can be purified and characterized using a variety of standard techniques that are well known to the skilled artisan. See, e.g. Ausubel et al. (1992), Bodanszky, et al. (1976) Peptide Synthesis, John Wiley and Sons, Second Edition, New York, New York and Zimmer et al. (1993) Peptides, pp. 393–394, ESCOM Science Publishers, B. V.

I.C. Nucleotide and Amino Acid Sequence Comparisons

The terms "identical" or percent "identity" in the context of two or more nucleotide or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using one of the sequence comparison algorithms disclosed herein or by visual inspection.

The term "substantially identical" in regards to a nucleotide or polypeptide sequence means that a particular sequence varies from the sequence of a naturally occurring sequence by one or more deletions, substitutions, or additions, the net effect of which is to retain at least some of biological activity of the natural gene, gene product, or sequence. Such sequences include "mutant" sequences, or sequences wherein the biological activity is altered to some degree but retains at least some of the original biological activity. The term "naturally occurring", as used herein, is used to describe a composition that can be found in nature as distinct from being artificially produced by man. For example, a protein or nucleotide sequence present in an organism, which can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory, is naturally occurring.

For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer program, subsequence coordinates are designated if necessary, and sequence algorithm program parameters are selected. The sequence comparison algorithm then calculates the percent sequence

identity for the designated test sequence(s) relative to the reference sequence, based on the selected program parameters.

Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman (1981) *Adv Appl Math* 2:482, by the homology alignment algorithm of Needleman & Wunsch (1970) *J Mol Biol* 48:443, by the search for similarity method of Pearson & Lipman (1988) *Proc Natl Acad Sci USA* 85:2444-2448, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, Madison, WI), or by visual inspection. See generally, Ausubel et al., 1992.

A preferred algorithm for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al. (1990) *J Mol Biol* 215: 403-410. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold. These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when the cumulative alignment score falls off by the quantity X from its maximum achieved value, the cumulative score goes to zero or below due to the accumulation of one or more negative-scoring residue alignments, or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the

alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength $W=11$, an expectation $E=10$, a cutoff of 100, $M=5$, $N=-4$, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix. See Henikoff & Henikoff (1989) *Proc Natl Acad Sci USA* 89:10915.

In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences. See, e.g., Karlin and Altschul (1993) *Proc Natl Acad Sci USA* 90:5873-5887. One measure of similarity provided by the BLAST algorithm is the smallest sum probability ($P(N)$), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a test nucleic acid sequence is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid sequence to the reference nucleic acid sequence is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

I.D. Antibodies

The present invention also provides an antibody that specifically binds a MS4A polypeptide. The term "antibody" indicates an immunoglobulin protein, or functional portion thereof, including a polyclonal antibody, a monoclonal antibody, a chimeric antibody, a single chain antibody, Fab fragments, and an Fab expression library. "Functional portion" refers to the part of the protein that binds a molecule of interest. In a preferred embodiment, an antibody of the invention is a monoclonal antibody. Techniques for preparing and characterizing antibodies are well known in the art (See, e.g., Harlow & Lane (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). A monoclonal antibody of the present invention can be readily prepared through use of well-known techniques such as the hybridoma techniques exemplified in U.S. Patent No 4,196,265 and the phage-displayed techniques disclosed in U.S. Patent No. 5,260,203.

The phrase "specifically (or selectively) binds to an antibody", or "specifically (or selectively) immunoreactive with", when referring to a protein or peptide, refers to a binding reaction which is determinative of the presence of the protein in a heterogeneous population of proteins and other biological materials. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein and do not show significant binding to other proteins present in the sample. Specific binding to an antibody under such conditions can require an antibody that is selected for its specificity for a particular protein. For example, antibodies raised to a protein with an amino acid sequence encoded by any of the nucleic acid sequences of the invention can be selected to obtain antibodies specifically immunoreactive with that protein and not with unrelated proteins.

The use of a molecular cloning approach to generate antibodies, particularly monoclonal antibodies, and more particularly single chain monoclonal antibodies, are also provided. The production of single chain antibodies has been described in the art. See, e.g., U.S. Patent No. 5,260,203. For this approach, combinatorial immunoglobulin phagemid libraries are prepared from RNA isolated from the spleen of the immunized animal, and phagemids expressing appropriate antibodies are selected by panning on endothelial tissue. The advantages of this approach over conventional hybridoma techniques are that approximately 10^4 times as many antibodies can be produced and screened in a single round, and that new specificities are generated by heavy (H) and light (L) chain combinations in a single chain, which further increases the chance of finding appropriate antibodies. Thus, an antibody of the present invention, or a "derivative" of an antibody of the present invention, pertains to a single polypeptide chain binding molecule which has binding specificity and affinity substantially similar to the binding specificity and affinity of the light and heavy chain aggregate variable region of an antibody described herein.

The term "immunochemical reaction", as used herein, refers to any of a variety of immunoassay formats used to detect antibodies specifically bound to a particular protein, including but not limited to competitive and

non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, *in situ* immunoassays (e.g.,
5 using colloidal gold, enzyme or radioisotope labels), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. See Harlow & Lane (1988) for a description of immunoassay formats and
10 conditions.

I.E. Protein Binding Assays

The term "binding" refers to an affinity between two molecules, for example, a ligand and a receptor. As used herein, "binding" means a preferential binding of one molecule for another in a mixture of molecules.
15 The binding of the molecules can be considered specific if the binding affinity is about $1 \times 10^4 \text{ M}^{-1}$ to about $1 \times 10^6 \text{ M}^{-1}$ or greater. Binding of two molecules also encompasses a quality or state of mutual action such that an activity of one protein or compound on another protein is inhibitory (in the case of an antagonist) or enhancing (in the case of an agonist). Exemplary
20 protein binding assays include but are not limited to Fluorescence Correlation Spectroscopy (FCS), Surface-Enhanced Laser Desorption/Ionization time-of-flight mass spectrometry (SELDI-TOF), and Biacore, each described further herein below.

Fluorescence Correlation Spectroscopy (FCS) measures the average
25 diffusion rate of a fluorescent molecule within a small sample volume (Madge et al. (1972) *Phys Rev Lett* 29:705-708; Maiti et al. (1997) *Proc Natl Acad Sci USA*, 94:11753-11757). The sample size can be as low as 10^3 fluorescent molecules and the sample volume as low as the cytoplasm of a single bacterium. The diffusion rate is a function of the mass of the molecule
30 and decreases as the mass increases. FCS can therefore be applied to protein-ligand interaction analysis by measuring the change in mass and therefore in diffusion rate of a molecule upon binding. In a typical

experiment, the target to be analyzed is expressed as a recombinant protein with a sequence tag, such as a poly-histidine sequence, inserted at the N-terminus or C-terminus. The expression takes place in *E. coli*, yeast or mammalian cells. The protein is purified using chromatographic methods.

5 For example, the poly-histidine tag can be used to bind the expressed protein to a metal chelate column such as Ni^{2+} chelated on iminodiacetic acid agarose. The protein is then labeled with a fluorescent tag such as carboxytetramethylrhodamine or BODIPYTM (Molecular Probes, Eugene, Oregon). The protein is then exposed in solution to the potential ligand, and

10 its diffusion rate is determined by FCS using instrumentation available from Carl Zeiss, Inc. (Thornwood, New York). Ligand binding is determined by changes in the diffusion rate of the protein.

Surface-Enhanced Laser Desorption/Ionization (SELDI) was developed by Hutchens & Yip (1993) *Rapid Commun Mass Spectrom* 7:576-

15 580). When coupled to a time-of-flight mass spectrometer (TOF), SELDI provides a means to rapidly analyze molecules retained on a chip. It can be applied to ligand-protein interaction analysis by covalently binding the target protein on the chip and analyzing by MS the small molecules that bind to this protein (Worrall et al. (1998) *Anal Biochem* 70:750-756). In a typical

20 experiment, the target to be analyzed is expressed as described for FCS. The purified protein is then used in the assay without further preparation. It is bound to the SELDI chip either by utilizing the poly-histidine tag or by other interaction such as ion exchange or hydrophobic interaction. The chip thus prepared is then exposed to the potential ligand via, for example, a

25 delivery system able to pipet the ligands in a sequential manner (autosampler). The chip is then washed in solutions of increasing stringency, for example a series of washes with buffer solutions containing an increasing ionic strength. After each wash, the bound material is analyzed by submitting the chip to SELDI-TOF. Ligands that specifically

30 bind the target are identified by the stringency of the wash needed to elute them.

Biacore relies on changes in the refractive index at the surface layer upon binding of a ligand to a protein immobilized on the layer. In this system, a collection of small ligands is injected sequentially in a 2-5 microliter cell, wherein the protein is immobilized within the cell. Binding is detected by surface plasmon resonance (SPR) by recording laser light refracting from the surface. In general, the refractive index change for a given change of mass concentration at the surface layer is practically the same for all proteins and peptides, allowing a single method to be applicable for any protein (Liedberg et al. (1983) *Sensors Actuators* 4:299-304; Malmquist (1993) *Nature* 361:186-187). In a typical experiment, the target to be analyzed is expressed as described for FCS. The purified protein is then used in the assay without further preparation. It is bound to the Biacore chip either by utilizing the poly-histidine tag or by other interaction such as ion exchange or hydrophobic interaction. The chip thus prepared is then exposed to the potential ligand via the delivery system incorporated in the instruments sold by Biacore (Uppsala, Sweden) to pipet the ligands in a sequential manner (autosampler). The SPR signal on the chip is recorded and changes in the refractive index indicate an interaction between the immobilized target and the ligand. Analysis of the signal kinetics of on rate and off rate allows the discrimination between non-specific and specific interaction.

I.F. Transgenic animals

It is also within the scope of the present invention to prepare a transgenic animal to mutagenize the MS4A locus or to express a transgene comprising nucleic acid sequences of the present invention. The term "transgenic animal", indicates an animal comprising a germline insertion of a heterologous nucleic acid. Transgenic animals of the present invention are understood to encompass not only the end product of a transformation method, but also transgenic progeny thereof.

The term "transgene", as used herein indicates a heterologous nucleic acid molecule that has been transformed into a host cell. For intended use in the creation of a transgenic animal, the transgene includes

genomic sequences of the host organism at a selected locus or site of transgene integration to mediate a homologous recombination event. A transgene further comprises nucleic acid sequences of interest, for example a targeted modification of the gene residing within the locus, a reporter gene,
5 or a expression cassette, each defined herein above.

Transgene integration can be used to create gene mutations, including "knock-out", "knock-in", or a "knock-down" mutations. Representative approaches are disclosed in the Examples presented below. The term "knock-out" refers to a homologous recombination event that
10 renders a gene inactive. Gene knock-out is generally accomplished by integration of the transgene at a chromosomal loci, thereby interrupting a gene residing at that loci. The term "knock-in" refers to *in vivo* replacement at a targeted locus. Knock-in mutations can modify a gene sequence to create a loss-of-function or gain-of-function mutation. The term "gene
15 knock-down" refers to a homologous recombination event wherein the transgene partially eliminates gene function. A knock-down animal can be created by transgenic expression of an antisense molecule, wherein a transgene comprising the antisense sequence and a relevant promoter are integrated into the genome at a non-essential loci. Expression of the
20 antisense or ribozyme molecule disrupts the corresponding gene function, although this disruption is generally incomplete (Luyckx et al. (1999) *Proc Natl Acad Sci U S A* 96(21):12174-12179).

Conditional mutation can be accomplished using transgenic methods in combination with the Cre-recombinase system in mice. Briefly, in one
25 instance, a transgenic mouse is derived that expresses Cre-recombinase under the direction of an inducible promoter. A second transgenic mouse bears a mutation of a gene of interest as well as a lox-P-flanked endogenous gene sequence. Such transgenic mice are mated, the resulting progeny having both the Cre-recombinase and lox-P-flanked transgenes. Induction
30 of Cre recombinase catalyzes excision of the lox-P-flanked transgene, thereby excising a portion of the endogenous gene sequence and revealing the mutated sequence. Conditional knockout can be varied according to the

temporal and spatial features of Cre recombinase expression, inherent in the selection of a promoter to drive Cre recombinase. See Postic et al. (1999) *J Biol Chem* 275(1):305-315; and Sauer (1998) *Methods* 14(4):381-392.

Transgenes can also be used for heterologous expression in a host
5 organism without generating phenotypically apparent mutations. By this method, nucleotide sequences of interest are introduced into the genome at a nonessential loci, whereby insertion alone does not disrupt an essential gene function. Optionally, expression of the transgene can generate a gain-of-function or ectopic function phenotype.

10 Techniques for the preparation of transgenic animals are known in the art. Exemplary techniques are described in U.S. Patent No. 5,489,742 (transgenic rats); U.S. Patent Nos. 4,736,866, 5,550,316, 5,614,396, 5,625,125 and 5,648,061 (transgenic mice); U.S. Patent No. 5,573,933 (transgenic pigs); 5,162,215 (transgenic avian species) and U.S. Patent No.
15 5,741,957 (transgenic bovine species). Briefly, nucleotide sequences of interest are cloned into a vector, and the construct is transformed into a germ cell. In the germ cell, a chromosomal rearrangement event takes place wherein the nucleic acid sequences of interest are integrated into the genome of the germ cell by homologous recombination. Fertilization and
20 propagation of the transformed germ cell results in a transgenic animal. Homozygosity of the mutation is accomplished by intercrossing.

I.G. Therapeutic Methods

The present invention further provides methods for discovering substances that can be used as pharmaceutical compositions. The term
25 "pharmaceutical composition" or "drug" as used herein, each refer to any substance having a biological activity. Substances discovered by methods of the present invention include but are not limited to polypeptide, proteins, peptides, chemical compounds, and antibodies.

A composition of the present invention is typically formulated using
30 acceptable vehicles, adjuvants, and carriers as desired.

Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution, and isotonic sodium chloride solution. In

addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or di-glycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectable compositions.

5 Injectable preparations, for example sterile injectable aqueous or oleaginous suspensions, are formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation can also be a sterile injectable solution or suspension in a nontoxic diluent or solvent, for example 1,3-butanediol.

10 A vector can be used as a carrier, for example an adenovirus vector, can be used for gene therapy methods. The vector is purified to sufficiently render it essentially free of undesirable contaminants, such as defective interfering adenovirus particles or endotoxins and other pyrogens such that it does not cause any untoward reactions in the individual receiving the vector
15 construct. A preferred means of purifying the vector involves the use of buoyant density gradients, such as cesium chloride gradient centrifugation.

 A transfected cell can also serve as a carrier. By way of example, a liver cell can be removed from an organism, transfected with a nucleic acid sequence of the present invention using methods set forth above and then
20 the transfected cell returned to the organism (e.g. injected intra-vascularly).

 Monoclonal antibodies or polypeptides of the invention can be administered parenterally by injection or by gradual infusion over time. Although the tissue to be treated can typically be accessed in the body by systemic administration and therefore most often treated by intravenous
25 administration of therapeutic compositions, other tissues and delivery means are provided where there is a likelihood that the tissue targeted contains the target molecule and are known to those of skill in the art.

 Representative antibodies for use in the present invention are intact immunoglobulin molecules, substantially intact immunoglobulin molecules,
30 single chain immunoglobulins or antibodies, those portions of an immunoglobulin molecule that contain the paratope, including antibody

fragments. It is within the scope of the present invention that a monovalent modulator can optionally be used.

Methods of preparing "humanized" antibodies are generally well known in the art, and can readily be applied to the antibodies of the present invention. Humanized monoclonal antibodies offer particular advantages over monoclonal antibodies derived from other mammals, particularly insofar as they can be used therapeutically in humans. Specifically, humanized antibodies are not cleared from the circulation as rapidly as "foreign" antigens, and do not activate the immune system in the same manner as foreign antigens and foreign antibodies.

With respect to the therapeutic methods of the present invention, a preferred subject is a vertebrate subject. A preferred vertebrate is warm-blooded; a preferred warm-blooded vertebrate is a mammal. A preferred mammal is a mouse or, most preferably, a human. As used herein and in the claims, the term "patient" includes both human and animal patients. Thus, veterinary therapeutic uses are provided in accordance with the present invention.

Also provided is the treatment of mammals such as humans, as well as those mammals of importance due to being endangered, such as Siberian tigers; of economical importance, such as animals raised on farms for consumption by humans; and/or animals of social importance to humans, such as animals kept as pets or in zoos. Examples of such animals include but are not limited to: carnivores such as cats and dogs; swine, including pigs, hogs, and wild boars; ruminants and/or ungulates such as cattle, oxen, sheep, giraffes, deer, goats, bison, and camels; and horses. Also provided is the treatment of birds, including the treatment of those kinds of birds that are endangered and/or kept in zoos, as well as fowl, and more particularly domesticated fowl, *i.e.*, poultry, such as turkeys, chickens, ducks, geese, guinea fowl, and the like, as they are also of economical importance to humans. Thus, provided is the treatment of livestock, including, but not limited to, domesticated swine, ruminants, ungulates, horses, poultry, and the like.

As used herein, the term "experimental subject" refers to any subject or sample in which the desired measurement is unknown. The term "control subject" refers to any subject or sample in which a desired measure is unknown.

5 As used herein, an "effective" dose refers to a dose(s) administered to an individual patient sufficient to cause a change in MS4A activity. One of ordinary skill in the art can tailor the dosages to an individual patient, taking into account the particular formulation and method of administration to be used with the composition as well as patient height, weight, severity of
10 symptoms, and stage of the biological condition to be treated. Such adjustments or variations, as well as evaluation of when and how to make such adjustments or variations, are well known to those of ordinary skill in the art of medicine.

A therapeutically effective amount can comprise a range of amounts.
15 One skilled in the art can readily assess the potency and efficacy of a MS4A modulator of this invention and adjust the therapeutic regimen accordingly. A modulator of MS4A biological activity can be evaluated by a variety of means including the use of a responsive reporter gene, interaction of MS4A polypeptides with a monoclonal antibody, analysis of cell subpopulations,
20 and measurement of $[Ca^{2+}]_i$ levels, each technique described herein.

Additional formulation and dose techniques have been described in the art, see for example, those described in U.S. Patent Nos. 5,326,902 and 5,234,933, and International Publication No. WO 93/25521.

For the purposes described above, the identified substances can
25 normally be administered systemically, parenterally, or orally. The term "parenteral" as used herein includes intravenous, intra-muscular, intra-arterial injection, or infusion techniques. Other compositions for administration include liquids for external use, and endermic liniments (ointment, etc.), suppositories, and pessaries which comprise one or more of
30 the active substance(s) and can be prepared by known methods.

II. CD20 Gene Family Members

II.A. Identification of CD20 Gene Family Members

The present invention provides MS4A nucleic acid and polypeptide sequences. Preferably, a MS4A gene comprises the sequence set forth as
5 any one of the odd-numbered SEQ ID NOs:1-37, a nucleic acid molecule that is substantially similar to any one of the odd-numbered SEQ ID NOs:1-37, or a nucleic acid molecule comprising a 20 base pair nucleotide sequence that is identical to a contiguous 20 base pair sequence of any one of the odd-numbered SEQ ID NOs:1-37.

10 To identify new CD20 gene family members, the human and mouse CD20 amino acid sequences (Tedder et al., 1988a; Tedder et al., 1988b) were used to search the translated GenBank databases, including expressed sequence tags, using the BLAST program (Altschul et al., 1997). Among 337 homologous sequences identified, at least 17 novel genes
15 expressed by mouse, human, and pig had predicted amino acid sequences homologous to CD20. Complete coding regions were predicted using overlapping nucleotide sequences obtained from sequenced ESTs and cDNAs that corresponded to unique, near full-length transcripts in humans and mice (Figure 1). All nucleotide sequences were verified by sequencing
20 multiple near full-length cDNAs isolated by applicants and 40 cDNAs obtained from the ATCC (American Tissue Culture Collection, Bethesda, Maryland, USA). In addition, a pig cDNA and its human counterpart homologous to CD20 were identified as GenBank submissions AJ236932.1 and AK000224, respectively. In total, unique cDNA clones were identified
25 that encode at least 16 distinct full-length CD20-like proteins.

Complete cDNA sequences encoding the human and mouse MS4A family members (*MS4A1*, *-A2*, *-A3*, *-A4A*, *-A5*, *-A6A*, *-A7*, *-A8B* and *-A12*) were also used to search the GenBank human genomic database (htgs; <http://www.ncbi.nlm.nih.gov/blast/>) using the BLAST program (Altschul et al.,
30 1997), as further described in Example 2. Two-hundred-twenty different contigs or distinct genomic DNA sequences were identified in the database of unfinished human genomic sequences that were either identical or similar

to MS4A family members. These sequences were predominantly derived from sixteen partially sequenced bacterial artificial chromosomes (BACs) that spanned 400-500 kb of human chromosome 11q12 (Table 1). Based on known cDNA sequences of MS4A family members, we were able to order and arrange these genomic sequences into overlapping continuous DNA segments. Since many of the contigs identified were overlapping, it was thereby possible to assemble long DNA sequences that encoded entire MS4A genes or portions of MS4A genes. Gaps between exon encoding DNA sequences were filled in many cases by additional sequence homology searches using DNA sequences found at the ends of gaps. When sequence differences were observed between different overlapping DNA fragments, consensus sequences were used or PCR primers were generated, that portion of genomic DNA was then amplified and sequenced to resolve ambiguous sequences.

BLAST analysis of the htgs phase 1 or phase 2 human genomic DNA sequences encoding MS4A cDNAs and the assembled and annotated human genomic sequence thereof, as disclosed herein, revealed the presence of each known human MS4A family member. In addition, three putative genes encoding unique MS4A family members were identified that localized to the q12-13.1 region of human chromosome 11. Complete coding regions were predicted using overlapping nucleotide sequences obtained from sequenced ESTs and cDNAs and by comparison of gene structure, described further herein below (Figure 2).

By identifying sequences that correlated with different MS4A genes in each BAC (Table 1), and by the assembly of minimal genomic DNA lengths that could encode each *MS4A* gene (Figure 2), we used the overlapping BACs to identify the order of the MS4A genes on chromosome 11q12 (Figure 6). This analysis also allowed us to determine the direction of gene transcription for most MS4A genes. Furthermore, the MS4A cDNA sequences, disclosed herein, were used to assemble genomic clones set forth as SEQ ID NOs:73-81. In some cases, multiple MS4A genes could be aligned within a continuous genomic sequence. For example, the genomic

sequence set forth as SEQ ID NO:77 comprises both the *MS4A4E* and *MS4A6A* genes. Similarly, the genomic region set forth as SEQ ID NO:79 comprises three *MS4A* genes: *MS4A7*, *MS4A5*, and *MS4A12*.

The *MS4A4E* gene encodes 660 bp of translated sequence (Figure 3), contained within at least seven exons (Figure 2). Exons were identified based on their sequence similarities with *MS4A4A* sequences and the identification of canonical splice-donor and -acceptor sites (Aebi & Weissmann, 1987). The *MS4A4E* gene sequence was at least 23,379 base pairs in length, if counted from the putative translation initiation ATG site until the TGA translation termination stop site (Figure 2). An exon encoding the putative 5' untranslated region of *MS4A4E*, was highly homologous with the corresponding sequence in *MS4A4A* cDNAs (disclosed herein). This sequence homology extended for >7 kbp upstream from this putative exon and also included upstream repetitive Alu elements. Representative upstream homologous sequences are shown in Figure 3. Similar sequence homologies were identified in the 3' untranslated regions of *MS4A4E* and *MS4A4A*, which extended beyond the poly-A attachment signal sequences (Figure 3). Based on the sequence similarities in translated and untranslated exons, it appears that the *MS4A4E* and *MS4A4A* genes resulted from a recent gene duplication event.

The *MS4A6E* gene encodes 441 bp of translated sequence (Figure 4), contained within at least four exons (Figure 2). Exons were identified based on their sequence similarities with *MS4A6A* cDNA sequences and the identification of canonical splice-donor and -acceptor sites (Aebi & Weissmann, 1987). In addition, the predicted gene sequences matched those found in three cDNA clones that were sequenced (ATCC Nos. 3704466, 1852248 and 3557769). The *MS4A6E* gene was at least 5,060 bp in length, if counted from the putative translation initiation ATG site until the TGA translation termination codon (Figure 2). The *MS4A6E* gene lacks exons that encode the first two membrane spanning domains present in most *MS4A* family proteins (Figures 2 and 7). An exon homologous with the 5' untranslated region of *MS4A6A* cDNAs was not identified within 7,629 bp

of sequence upstream of the exon encoding the translation initiation site of *MS4A6E*. However, there was a canonical 3' splice region upstream of the ATG initiation codon located at identical positions in the *MS4A6E* and *MS4A6A* genes. Similar sequence homologies were identified in the 3' untranslated regions of *MS4A6E* and *MS4A6A* that extend beyond the sequence shown in Figure 4. Based on the sequence similarities in translated and untranslated exons, it appears that the *MS4A6E* and *MS4A6A* genes represent a recent gene duplication event, although several exons encoding translated sequence were lost in the *MS4A6E* gene (Figure 2).

The *MS4A10* gene encodes 726 bp of translated sequence (Figure 5), contained within at least six exons (Figure 2). Exons were identified based on their sequence similarities with mouse *MS4a10* cDNA sequences and the identification of canonical splice-donor and -acceptor sites (Aebi & Weissmann, 1987). The *MS4A10* gene was at least 8,183 bp in length if counted from the putative translation initiation ATG site until the TGA translation termination stop site (Figure 2). An exon homologous with the 5' untranslated region of mouse *MS4a10* cDNAs was not identified within 8,829 bp of sequence upstream of the exon encoding the translation initiation site of *MS4A10*. However, there was a canonical 3' splice region upstream of the ATG initiation codon located at identical positions in the *MS4A10* and *MS4a10* genes. Modest sequence homologies were identified in the 3' untranslated regions of *MS4A10* and *MS4a10* (Figure 5).

Table 1
Human BACs Containing MS4A Genes

BAC	Accession No. ^a	Chromosome	MS4A gene ^b
RP11-206B10	AC009703	15	A4A, A4E, A6A
RP11-21B14	AC013807	unknown	A6A, A2, A3
RP11-24D1	AC015840	unknown	A4A, A5, A6E, A7
RP11-652L5	AC018966	11	A4A, A4E, A6A
RP11-448N3	AC024066	11	A8B
RP11-312N17	AC027599	11	A8B, A10
RP11-196E16	AC027787	15	A5, A1
CMB9-79B2	AP000748	11q23	A10
RP11-804A23	AP000777	11	A10
RP11-736I10	AP000790	11q12	A3
RP11-804B24	AP000934	11	A10
RP11-729B4	AP001034	11q12	A5, A12, A1
CMB9-2M23	AP001181	11q12	A2, A3
CMB9-100I1	AP001257	11q12	A6A, A4E
CMB9-49F18	AP001259	11	A8B
RP11-68H20	AP001986	11q	A10

^aGenBank Accession number for the indicated BAC.

5 ^bindicates the MS4A gene sequences that mapped to each BAC.

II.B. MS4A Nomenclature

In collaboration with the Human Gene Nomenclature Committee (www.gene.ucl.ac.uk/nomenclature/), this gene family was designated as the MS4A family (Membrane Spanning 4-domain family, subfamily A). The MS4 designation is to accommodate the future identification of genes encoding proteins with a similar structure, yet with unresolved functions. Subfamily A will designate the CD20 family. Using this nomenclature, the CD20 gene was designated as *MS4A1*, *FcεRIβ* as *MS4A2*, and HTm4 as *MS4A3*. Among the 16 novel genes identified, 8 human genes were named *MS4A4A*, *MS4A4E*, *MS4A5*, *MS4A6A*, *MS4A6E*, *MS4A7*, *MS4A8B*, and *MS4A12*. A ninth gene encoded a protein homologous with the single member of the mouse MS4a10 subfamily. This gene was tentatively designated as *MS4A10*. The remaining genes were of mouse or pig origin and were therefore labeled as *MS4a3-MS4a12* based on the nomenclature of homologous genes corresponding to human counterparts. Distinct mouse genes that encoded proteins with highly homologous sequences were designated as *MS4a4B*, *MS4a4C*, *MS4a4D*, and as *MS4a6B*, *MS4a6C*, and *MS4a6D* to signify close homology.

II.C. MS4A Gene Chromosome Locations

Chromosome locations for the human *MS4A4A*, *MS4A6A*, *MS4A7*, and *MS4A8B* genes were identified in two distinct homology searches. Regions of human *MS4A4A* (bp 1286-1588), *MS4A6A* (bp 682-1106), *MS4A7* (bp 502-941), *MS4A7* (bp 1015-1177), and *MS4A8B* (bp 1007-1350), were 98%, 98%, 97%, 99% and 97% identical with human STS genomic sequence tag sites, WI-11578, SHGC-36634, WI-12101, WIAF-3856, and WI-14145, respectively (<http://www.ncbi.nlm.nih.gov/blast>). These genomic sequence tag sites are located on human chromosome 11 at Genomic Database locus D11S1357-D11S913, which maps to 11q12-13 (<http://www.ncbi.nlm.nih.gov/genemap>). These mapping results were confirmed using the UniGene collection at the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/Genemap98/>) for expressed sequence tags identical to human *MS4A4A*, *MS4A6A*, *MS4A7*,

MS4A8B sequences. By this analysis, at least 7 of the 9 currently identified human *MS4A* genes are clustered.

The organization of the 12 *MS4A* genes on human chromosome 11 was determined by identifying sequenced human genomic DNA fragments (contigs of different lengths) from 15 BAC clones (Table 1). Contiguous DNA segments for each BAC were constructed based on human *MS4A* exon and cDNA sequences, and overlapping contigs. Although some gaps were present in *MS4A* gene introns (Figure 2) or between *MS4A* genes, the relative position of each gene on chromosome 11q12-13.1 was determined (Figure 6). *MS4A1* was located in a telemetric region of 11q12-13.1 compared with *MS4A2* and *MS4A3*. Seven *MS4A* genes were located in between *MS4A1* and *MS4A2*. Two other *MS4A* genes, *MS4A8B* and *MS4A10* were centromeric to *MS4A2* and *MS4A3*, although the distance between these genes was not determined. Interestingly, *MS4A6A*, *MS4A4E*, *MS4A4A* and *MS4A6E* were arranged linearly suggesting that these genes might have arisen through the duplication of a single genomic element. It is envisioned that this genetic locus extends further and contains additional *MS4A* genes.

II.A. *MS4A* Gene Structure

Complete coding region sequences were verified for each deduced protein, except for the *MS4a3* cDNA that was not full-length (Figure 1). Proposed ATG translation initiation codons were based on the translation initiation consensus sequence, ANNATG (Kozak (1986) *Cell* 44:283-292), and the existence of in-frame upstream translation stop codons in most cases. Whether the first or second ATG codon in mouse *MS4a8B* was used for translation initiation was unknown although the second ATG was identical with the start codon of human *MS4A8B* (Figure 7).

Poly(A) attachment signal sequences were identified in the proximal 3' untranslated regions of each gene product except *MS4A6A*, *MS4A6E*, *MS4A10*, and *MS4a6C*. Two poly(A) signal sequences were found in *MS4a4D*, *MS4A5*, and *MS4a10* transcripts, while four were observed in *MS4A4A* transcripts.

The disclosed MS4A cDNAs were further used to annotate the genomic sequence derived from BAC clones. Annotated features include definition of coding regions, intron|exon junctions, sequences upstream of the initial coding region of each gene that comprise the promoter region, and
5 other adjacent sequences that could also comprise gene regulatory elements. Representative methods for further characterizing a MS4A promoter region are disclosed in Example 9.

Annotation of human MS4A genomic regions (SEQ ID NOs:73-81), as disclosed herein, enabled a comparison of gene structure among MS4A
10 genes. The overall domain organization of each MS4A gene was similar (Figures 2 and 7). All exon|intron|exon boundaries were consistent with consensus splice-donor and -acceptor sequences unless otherwise indicated, with exon|GTGAGT-intron-CAG|exon sequences in most cases (Aebi & Weissmann, 1987). In addition, the splice junctions for all translated
15 exons were located after the third nucleotide in each codon. Most MS4A proteins were encoded by 6 exons except MS4A2, MS4A5, and MS4A6E (Figure 2 and 7). In these exceptions: the N-terminal cytoplasmic domain of MS4A2 was encoded by two exons (Küster et al., 1992); the *MS4A5* and *MS4A6E* genes did not encode C-terminal cytoplasmic domains; and the
20 *MS4A6E* gene had only two membrane spanning domains. Intron lengths demonstrated wide variation from 181 bp in *MS4A12* to 13,731 bp in *MS4A5*. In some cases however, exact intron lengths were not determined; *MS4A3*, *MS4A4*, and *MS4A12* (Figure 2). Distances between translation initiation and termination codons were determined for most MS4A genes;
25 with *MS4A6E* being the smallest (5,060 bp) and *MS4A4E* being the longest (23,379 bp) genes (Figure 6). Thus, the intron|exon organization of all MS4A family members is consistent with the high degree of conservation within this gene family.

There were no amino-terminal signal sequences, although all MS4A
30 proteins contained hydrophobic regions of sufficient length to pass through the membrane at least four times. Notable was a marked clustering of charged residues at both ends of the putative transmembrane domains,

some of which were highly conserved. In some cases, the first and second putative transmembrane domains of MS4A proteins were a continuous stretch of hydrophobic amino acids without an obvious inter-transmembrane hydrophilic bridge. By contrast, MS4A4A and MS4A7 had 6 to 7 hydrophilic amino acids inserted between the first and second hydrophobic domains. In human MS4A4A and mouse MS4a4B, MS4a4C, and MS4a4D, an extensive hydrophobic region followed the fourth putative membrane-spanning domain. Thus, the overall structure of MS4A family members was well conserved.

10 II.E. MS4A Gene Splice Variants

Among the MS4A cDNAs sequenced and EST sequences analyzed, multiple splice variants were identified that encoded variant MS4A proteins. In most cases, exons were spliced out, which generated truncated protein products. Potential splice variants of the *MS4A4A*, *MS4A5*, *MS4A6A*, and *MS4A7* genes were identified. Whether these alternatively spliced variants produce functional proteins has yet to be determined.

Two splice variations of the *MS4A4A* gene were identified during an analysis of *MS4A4A* mRNA expression by lymphoblastoid cell lines. Most of the hematopoietic cell lines examined expressed transcripts encoding a full-length MS4A4A protein as shown in Figure 7. However, a second smaller transcript was also expressed in most cases that contained a potential exon deletion of 158 nucleotides. This was a frequent event since 40% of *MS4A4A* cDNAs generated from the BJAB B cell line encoded the truncated protein. In addition, the same splicing event was observed in two of five EST sequences that covered this region of the MS4A4A protein. Splicing-out this potential exon deleted the third membrane-spanning domain and the second extracellular loop from the full-length protein (positions 110-163, Figure 3). Of interest, this splicing event fused the first/second membrane spanning domains with the fourth membrane spanning domain. However, the fourth transmembrane spanning domain in MS4A4A is followed by another hydrophobic region of sufficient length to traverse the membrane (disclosed herein). This suggests that differential splicing can generate an

alternative MS4A4A protein with four membrane spanning domains lacking a significant extracellular domain.

In the case of the *MS4A5* gene, two of nine *MS4A5* EST sequences analyzed (GenBank Accession Nos. AA411806 and AA781801) encoded a splice variant that preserved the reading frame of the transcript. In both sequences, the exon encoding the third membrane-spanning domain and the second extracellular loop from the full-length protein (TM3, Figure 1) was spliced out using normal splice-donor and -acceptor sequences, which deleted 51 amino acids (114-164) from the full length protein (Figure 7). This deletion resulted in a protein with the first/second membrane spanning domains fused with the fourth predicted membrane-spanning domain. Thus, the truncated MS4A5 protein would possess three membrane-spanning domains with an extracellular carboxyl-terminal domain.

A novel splicing event was observed in the *MS4A6A* gene which resulted in a truncated protein. A novel splice donor site (CAG T⁶⁸³ | GT GAG T) is located within the exon encoding the TM3/extracellular loop domains (Figure 4). This cryptic splice donor site was spliced with the normal 3' splice acceptor site of the exon encoding the TM4 domain, which thereby deletes nucleotides 684-787 from *MS4A6A* transcripts (Figure 4). Since there was an extra T introduced into the codon sequence due to this alternative splicing event, there was a frameshift in the coding sequence. This potentially results in the attachment of a novel 30 amino acid sequence (-WNSLSDADLHSAGILPSCAHCCAAVETGLL) that is not predicted to be hydrophobic. Thus, the variant MS4A protein would be 70 amino acids shorter and would lack the fourth membrane-spanning and cytoplasmic domains. This alternative splicing event was found in 3 of 29 EST sequences that encoded this region (GenBank Accession Nos. AI278475, AA461046, and AA448335) and in one cDNA clone (GenBank Accession No. AB013104).

Splice variation in *MS4A7A* transcripts produces two distinct protein products in addition to the presumably normal protein. In one case, a splice variation in *MS4A7A* transcripts produces a protein product similar in

structure to the MS4A6E protein. The exon encoding the first/second membrane spanning domains (amino acids 50-94, Figure 7) was deleted in 2 of 4 MS4A7 EST sequences analyzed (GenBank Accession Nos. N42191 and R11179) that cover this region. Thus, the protein product would have a longer N-terminal cytoplasmic domain and only two membrane spanning domains. In the second case, the exon encoding the fourth membrane-spanning domain (amino acids 183-216) was deleted in 2 EST sequences (GenBank Accession Nos. R11180 and A188478) out of 18 sequences analyzed (Figure 7).

10 II.F. MS4A Gene Polymorphisms

Putative polymorphisms were identified in the MS4A6A gene. Two nucleotide substitutions were found in cDNA clone ATCC No. 499181 and in 13 of 38 EST sequences analyzed (Figure 1). The first substitution was at nucleotide 373 that exchanged a C for a T, which did not alter the amino acid sequence. The second substitution resulted in a Ser in place of Thr at amino acid 185. In addition, a third substitution was found in 4 of the 38 EST sequences analyzed where a Ser was substituted in place of an Ala at amino acid position 183. This substitution was paired with a Ser to Thr substitution at amino acid position 185 in half of the clones analyzed. These differences most likely represent common sequence polymorphisms since they were observed in multiple independent cDNA clones. Based on our genetic DNA analysis, it is unlikely that these differences could represent transcripts from distinct genes that are almost identical in coding sequence.

As with the MS4A6A gene (disclosed herein), potential gene polymorphisms were observed in MS4A6E. Three cDNA clones representing partial transcripts were sequenced completely on both strands. The predicted MS4A6E gene product and one cDNA clone (ATCC No. 3704466) had identical sequences. However, the ATCC No. 3557769 cDNA had a nucleotide substitution at position 314 (Figure 4) that exchanged a T for a C, which did not alter the predicted amino acid sequence. The ATCC No. 1852248 cDNA clone had the longest insert that starts at nucleotide position 60 and ended at position 661 as shown in Figure 4. This cDNA had

a substitution at nucleotide 153 that exchanged a G for a T, which resulted in a Phe in place of Val at amino acid 47 (Figure 4). Therefore, sequence polymorphisms can exist within the *MS4A6E* gene.

Other potential polymorphisms were observed in other MS4A family members based on consistent nucleotide variations found in *MS4A4E* sequences.

The assembly and annotation of genomic sequences comprising MS4A genes in the region of human chromosome 11q12-13.1, disclosed herein for the first time, provide source material for identification of polymorphisms that are linked to MS4A genes. Such polymorphisms can include single nucleotide polymorphisms as disclosed within the *MS4A6A* and *MS4A6E* coding region sequences. In addition, polymorphisms within or genetically linked to MS4A genes can also comprise restriction length polymorphisms (RFLPs) (Lander & Botstein (1989) *Genetics* 121:185-199), short tandem repeat polymorphisms (STRPs), short sequence length polymorphisms (SSLPs) (Dietrich et al. (1996) *Nature* 380:149-152), amplified fragment length polymorphisms (AFLPs) (Latorra et al. (1994) *PCR Methods Appl* 3(6):351-358), and microsatellite markers (Schalkwyk et al. (1999) *Genome Res* 9:878-887). Identification of polymorphisms within an isolated DNA molecule are known to one of skill in the art.

II.G. MS4A Proteins

The MS4A genes encoded proteins of 16-29 kDa (Table 2).

Table 2
MS4A Family Members

Human		Mouse		Human/Mouse
Name	kDa	Name	kDa	Homology
		MS4a3		63% (partial)
MS4A4A	23			
		Ms4a4B	24	41%
		Ms4a4C	24	44%
		Ms4a4D	24	40%
MS4A4E	24			
MS4A5	22			
MS4A6A	27			
		Ms4a6B	27	52%
		Ms4a6C	24	51%
		Ms4a6D	26	53%
MS4A6E	16			
MS4A7	26	MS4a7	26	53%
MS4A8B	26	MS4a8B	29	63%
MS4A10	27	MS4a10	29	52%
MS4A12	26	MS4a12(pig)	26	60%

^aPredicted molecular weights for the new MS4A family members and the
5 percentage amino acid sequence identity between deduced MS4A and
MS4a proteins.

Comparisons between CD20 and the predicted amino acid sequences for human MS4A4A, MS4A5, MS4A6A, MS4A7, MS4A8B, and MS4A12 revealed 23-29% amino acid sequence identity (Figure 7). The highest degree of identity was found in the first three transmembrane domains with multiple regions of conserved amino acids. In particular, the amino acid sequences LGAXQI (SEQ ID NO:57) and LSLG (SEQ ID NO:58) were common within the first transmembrane domain, GYPFWG (SEQ ID NO:60) and FIISGSLS (SEQ ID NO:61) were common in the second domain, and SLX₂NX₂SX₃AX₂G (SEQ ID NO:62) was found in the third transmembrane domain. The first and second transmembrane domains of MS4A8B were 46% identical in amino acid sequence with human CD20, 41% identical with FcεRIβ, and 39% identical with HTm4. The MS4A4A, MS4A5, MS4A6A, and MS4A7 proteins were most homologous in their first and second transmembrane domains with the human FcεRIβ chain, with 37-46% amino acid sequence identity. There was large variation between MS4A proteins in the N- and C-terminal cytoplasmic domains. However, Pro residues were significantly over-represented within the N- and C-terminal cytoplasmic domains of most MS4A family members. There was some sequence identity in the first potential extracellular loop that was ~13 amino acids in length for each protein. By contrast, the second predicted extracellular loop ranged from 10-46 amino acids in length with diverse sequences.

The putative *MS4A4E* gene encodes a 220 amino acid protein of 23.8 kDa with a predicted amino acid sequence that is 76% identical with the MS4A4A protein (Figure 3). Consistent with other MS4A proteins, the most significant homologies between MS4A4E and other MS4A family members were found in the membrane spanning domains (Figure 7). Common amino acid motifs were readily visualized such as KXLGAIQI (SEQ ID NO:57), GYPXWG (SEQ ID NO:60), and SGXLSI (SEQ ID NO:59) in the first and second hydrophobic regions that represent potential transmembrane regions. The intracellular N- and C-terminal domains were highly conserved

between MS4A4E and MS4A4A, but were divergent from other family members.

The putative *MS4A6E* gene encodes a 147 amino acid protein of 15.9 kDa with a predicted amino acid sequence that is 78% identical with the
5 MS4A6A protein (Figure 4). The most significant homologies between MS4A6E and other MS4A family members were found in the membrane spanning domains, although MS4A6E only had two (TM3 and TM4) membrane-spanning domains (Figures 4 and 7). The putative second
10 4). Common amino acid motifs were readily visualized in the hydrophobic regions that represent potential transmembrane regions. The intracellular N-terminal domain was highly conserved between MS4A6E and MS4A6A, but were divergent from other family members. MS4A6E protein also lacks a C-terminal cytoplasmic domain (Figure 4).

15 The putative *MS4A10* gene encodes a translated 241 amino acid protein of 26.9 kDa with a predicted amino acid sequence that is 52% identical with the mouse MS4a10 protein (Figure 5). The most significant homologies between MS4A10 and MS4a10 were found in the membrane spanning domains and the putative second extracellular loop (Figure 5).
20 Although the N-terminal cytoplasmic domains of MS4A10 and MS4a10 were of similar length, the intracellular N- and C-terminal domains had the lowest sequence homologies among domains. The cytoplasmic C-terminal domain was 28 amino acids shorter in MS4A10 than MS4a10. Nonetheless, based on the sequence similarities of translated regions, it appears that MS4A10
25 and MS4a10 represent homologous genes that are more similar to one another than other MS4A family members.

Ten novel mouse MS4A proteins were identified that shared 40-63% amino acid sequence identity with their potential human counterparts (Figure 7, Table 2). For comparison, the mouse and human CD20 proteins are 74%
30 identical in amino acid sequence (Tedder et al., 1988a). A single partial cDNA was identified that encoded the mouse homologue for HTm4 (*MS4a3*, Figure 7). The predicted amino terminus of the proposed MS4a3 protein

was 23 amino acids shorter than in the human protein, although their overlapping regions were 63% identical in amino acid sequence. In all cases, the transmembrane domains of the human and mouse MS4A proteins were the most well conserved regions. For example, the human
5 MS4A8B protein was 78% identical in sequence to MS4a8B in the first 3 transmembrane domains and 68% identical in domain 4. Additional MS4A genes are likely to be identified in humans and mice, including the mouse MS4A5 homologue.

A UPGMA (unweighted pair group method using arithmetic averages)
10 tree showing relatedness of deduced MS4A and MS4a protein sequences is depicted in Figure 8.

III. Methods for Detecting a MS4A Nucleic Acid Molecule

In another aspect of the invention, a method is provided for detecting
15 a nucleic acid molecule that encodes a MS4A polypeptide. According to the method, a biological sample having nucleic acid material is procured and hybridized under stringent hybridization conditions to a MS4A nucleic acid molecule of the present invention. Such hybridization enables a nucleic acid molecule of the biological sample and the MS4A nucleic acid molecule to
20 form a detectable duplex structure. Preferably, the MS4A nucleic acid molecule includes some or all nucleotides of any one of the odd-numbered SEQ ID NOs:1-37. Also preferably, the biological sample comprises human nucleic acid material.

III.A. Expression of MS4A Family Members in Hematopoietic Cells

25 Since CD20, Fc ϵ RI β , and HTm4 expression are restricted to hematopoietic tissues, MS4A gene transcription was assessed by PCR amplification of cDNA from eleven human hematopoietic cell lines. Like CD20, MS4A8B was only expressed by B cell lines (Table 3). MS4A5 was only expressed by a promonocytic cell line. MS4A6A transcripts were
30 expressed by B cell, myelomonocytic, and erythroleukemia cell lines. MS4A4A mRNA was expressed by all cell lines examined, although the relative mRNA levels varied significantly. MS4A7 was expressed in most,

but not all of the cell lines tested. *MS4A12* transcripts were not detected in these cell lines. Thus, most *MS4A* family members are likely to be expressed in hematopoietic tissues.

ESTs encoding *MS4A* transcripts were isolated from a variety of
5 different cDNA libraries. *MS4A4A* ESTs were from aorta, brain, breast,
heart, kidney, lung, ovary, pancreas, placenta, prostate, stomach, testis, and
uterine tissues. *MS4A5* ESTs were only isolated from testis. *MS4A6A* ESTs
were from aorta, brain, the central nervous system, colon, gall bladder,
heart, kidney, lung, muscle, ovary, pancreas, placenta, prostate, skin,
10 stomach, tonsil, uterus and embryonic tissues. *MS4A7* ESTs were from
lung, kidney, lymphocytes, mammary gland, placenta, spleen, testis, thymus,
and uterine tissues. *MS4A8B* ESTs were from brain, lung, uterus and
embryonic tissues. A single *MS4A12* EST was isolated from colon. This
demonstrates differential *MS4A* gene transcription among lymphoid and
15 non-lymphoid tissues.

Table 3
MS4A mRNA Expression by Human Lymphoblastoid Cell Lines

<u>MS4A family member^a</u>										
Cell lines:	1	2	3	4A	5	6A	7	8B	12	G3PDH
Pre-B:										
NALM-6	-	-	-	+++	-	-	-	-	-	+++
B cell:										
BJAB	+++	-	-	+++	-	-	+++	+	-	+++
DAUDI	+++	-	-	+	-	-	+++	+	-	+++
SB	+++	-	-	++	-	+++	+++	+	-	+++
T cell:										
HSB-2	-	-	-	+	-	-	-	-	-	+++

HUT-78	-	-	-	+	-	-	+	-	-	+++
JURKAT	-	-	-	+	-	-	-	-	-	+++
MOLT15	-	-	-	+	-	-	++	-	-	+++
Myelomonocyte:										
HL60	-	-	+++	++	-	+++	+++	-	-	+++
U937	-	-	+++	+++	+	+	+++	-	-	+++
Erythroleukemia:										
K562	-	+	+++	+++	-	+	-	-	-	+++

^aGene transcription was assessed by PCR amplification of cDNA generated from mRNA isolated from each cell type. Values represent the level of PCR product generated relative to the glyceraldehyde-3-phosphate dehydrogenase (G3PDH) control in the separate PCR reactions: -, no specific PCR product detected; +, low levels of the appropriate band were detectable; ++ to +++ appropriate bands of increasing intensity were readily visualized in all samples examined. Identical results were obtained using the different primer pairs for cDNA amplification.

Since most of the MS4A genes are expressed by hematopoietic cells, *MS4A4E*, *MS4A6E* and *MS4A10* transcription were assessed by RT-PCR amplification of cDNA from human hematopoietic cell lines and human tissues. Transcripts from eleven human hematopoietic cell lines were
5 evaluated; one pre-B cell line (NALM-6), three B cell lines (BJAB, DAUDI, and SB), four T cell lines (HSB-2, HUT-78, JURKAT, and MOLT15), two myelomonocytic lines (HL60 and U937), and one erythroleukemia cell line (K562). In addition, transcripts from eight human tissues were evaluated; colon, ovary, peripheral blood leukocytes, prostate, small intestine, spleen,
10 testes and thymus. However, *MS4A4E*, *MS4A6E* and *MS4A10* transcripts were not detected in any of these cell lines or tissues.

MS4A4E, *MS4A6E*, and *MS4A10* sequences were also used to search the translated GenBank databases using the BLAST program (Altschul et al., 1997). Eleven EST sequences representing *MS4A6E*
15 transcripts were found that represented nine cDNAs isolated from pooled fetal organ libraries (GenBank Accession Nos. AA382998, AA909515, AA917066, AI222355, AI279944, AI684553, AI699419, AI743473, AI806247), one cDNA from a pooled germ cell tumor library (GenBank Accession No. AI968835), and one cDNA from a colon tumor (GenBank
20 Accession No. AW951636). EST cDNAs encoding *MS4A4E* or *MS4A10* sequences were not identified. This suggests that *MS4A4E*, *MS4A6E*, and *MS4A10* transcripts are rare among normal tissues or they are primarily expressed during oncogenesis or embryogenesis.

MS4a gene expression by mouse tissues was assessed by Northern
25 analysis and PCR amplification of cDNAs (Table 4). In most cases assessed, Northern analysis failed to detect specific MS4a transcripts in tissues that revealed transcript production by PCR amplification. These results suggest that MS4a transcripts are only produced by subpopulations of cells within each tissue such that transcript levels were often below the
30 level of detection by Northern analysis. Nonetheless, *MS4a4B*, *MS4a4C*, and *MS4a6B* transcripts were found at high levels in thymus, spleen and peripheral lymph nodes, with less abundant levels in non-lymphoid tissues.

MS4a6C was only expressed by thymus, spleen, PLN and bone marrow. *MS4a4C*, *MS4a6D* and *MS4a7* were expressed in all tissues examined. *MS4a8B* transcripts were expressed by spleen, peripheral lymph nodes, colon, liver, heart, lung and bone marrow. *MS4a10* transcripts were found in

5 thymus, kidney, colon, brain, and testis. In addition, CD20 (*MS4a1*), FcεRIβ (*MS4a2*), and *MS4a3* expression were primarily restricted to hematopoietic tissues. *MS4a3*, *MS4a4B*, *MS4a4C*, *MS4a6B*, *MS4a6C*, *MS4a6D*, *MS4a7*, *MS4a8B*, and *MS4a10* were also expressed by various hematopoietic and lymphoblastoid cell lines. Therefore, most *MS4a* family members were

10 expressed by hematopoietic cells.

Table 4
MS4a Gene Expression by Mouse Tissues^a

MS4a	Thymus	Spleen	PLN	BM	Liver	Kidney	Heart	Colon	Lung	Brain	Teste
1	+	+++	+++	+	-	-	-	-	+	-	-
2	+	+	+	+++	-	+	-	-	+	-	-
3	+	+	+	+++	-	-	-	-	+	+	-
4B	+++	+++	+++	++	+	+	+	+	+	-	-
4C	+++	+++	+++	+++	+	+	+	+	+	+	+
4D	+	+	++	-	+	+	++	++	++	-	+
6B	+++	+++	+++	++	+	-	+	+	+	-	++
6C	+	+	+	++	-	-	-	-	-	-	-
6D	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	+++
7	++	++	++	++	+	+	+	++	+	+	+
8B	-	+	+	+	+	-	+	++	+	-	-
10	+	-	-	-	-	+	-	+	-	+	++
G3PDH	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

^aGene transcription was assessed by PCR amplification of cDNA generated from mRNA isolated from tissue samples. Values represent the level of PCR product generated relative to the glyceraldehyde-3-phosphate dehydrogenase (G3PDH) control as described for Table 3. Peripheral lymph node (PLN) and bone marrow (BM).

15

Expression of MS4A family members was also assessed in mouse hematopoietic cell lines (Table 5). Nine of the twelve MS4A genes were expressed in pre-B cell lines and five of the MS4A genes were expressed in B cell lines. Six of the MS4A genes were expressed by T cell lines. These data suggest that B cells can express most members of the MS4A gene family, although the patterns of expression of each gene is distinct.

Table 5MS4a Expression by Mouse Lymphoid Tissues and Cell Lines^a

	<u>Tissues</u>		<u>Pre B cell lines</u>			<u>B cell lines</u>		<u>T cell lines</u>	
MS4a	Spleen	Thymus	300.19	38B9	70Z	A20	AJ9	BW514	EL-14
1	+++	+	-	-	-	+++	+++	-	-
2	+	+	-	-	+	-	-	-	-
3	+	+	-	+	-	-	-	-	-
4B	+++	+++	-	-	-	-	-	-	++
4C	+++	+++	-	+	+	++	-	-	+
4D	+	+	-	-	-	-	-	-	-
6B	+++	+++	-	+	+++	+	-	+++	+++
6C	+	-	-	+	+	-	-	-	+
6D	+++	+++	-	++	+++	-	+	-	+++
7	++	++	-	-	++	-	-	-	-
8B	+	-	-	-	++	-	-	-	-
10	-	+	-	-	+	+	+	+	-
G3PDH	+++	+++	+++	+++	+++	+++	+++	+++	+++

10

^aGene transcription was assessed by PCR amplification of cDNA generated from mRNA isolated from each cell type. Values represent the level of PCR product generated relative to the glyceraldehyde-3-phosphate dehydrogenase (G3PDH) control in three separate PCR reactions: -, no specific PCR product detected; +, low levels of the appropriate band were detectable; ++ to +++, appropriate bands of increasing intensity were readily visualized in all samples examined. Identical results were obtained using two different primer pairs for cDNA amplification.

15

III.B. Detection of MS4A Polymorphisms

In another embodiment, genetic assays based on nucleic acid molecules of the present invention can be used to screen for genetic variants by a number of PCR-based techniques, including single-strand
5 conformation polymorphism (SSCP) analysis (Orita, M., et al. (1989) *Proc Natl Acad Sci USA* 86(8):2766-2770), SSCP/heteroduplex analysis, enzyme mismatch cleavage, and direct sequence analysis of amplified exons (Kestila et al. (1998) *Mol Cell* 1(4):575-582; Yuan et al. (1999) *Hum Mutat* 14(5):440-446). Automated methods can also be applied to large-scale
10 characterization of single nucleotide polymorphisms (Brookes (1999) *Gene* 234(2):177-186; Wang et al. (1998) *Science* 280(5366):1077-82). The present invention further provides assays to detect a mutation of a variant MS4A locus by methods such as allele-specific hybridization (Stoneking et al. (1991) *Am J Hum Genet* 48(2):370-82), or restriction analysis of amplified
15 genomic DNA containing the specific mutation.

IV. Recombinant Production of a MS4A Polypeptide

The present invention also provides a method for recombinant production of a MS4A polypeptide, as described in Example 3. Preferably,
20 the recombinant polypeptide comprises some or all of the amino acid sequences of any one of the even-numbered SEQ ID NOs:2-38.

Recombinantly produced proteins are useful for a variety of purposes, including structural determination of a MS4A polypeptide, generation of an antibody that recognizes a MS4A polypeptide, and screening assays to
25 identify a chemical compound or peptide that interacts with a MS4A polypeptide, described further herein below.

V. Production of MS4A Antibodies

In another aspect, the present invention provides a method of
30 producing an antibody immunoreactive with a MS4A polypeptide, the method comprising recombinantly or synthetically producing a MS4A polypeptide, or portion thereof, to be used as an antigen. The MS4A

polypeptide is formulated so that it can be used as an effective immunogen. An animal is immunized with the formulated MS4A polypeptide, generating an immune response in the animal. The immune response is characterized by the production of antibodies that can be collected from the blood serum of the animal. Optionally, cells producing a MS4A antibody can be fused with myeloma cells, whereby a monoclonal antibody can be selected. Exemplary methods for producing a monoclonal antibody that recognizes a MS4A protein are described in Example 4. Preferred embodiments of the method use a polypeptide set forth as any one of the even-numbered SEQ ID NOs:2-38.

The present invention also encompasses antibodies and cell lines that produce monoclonal antibodies as described herein.

The foregoing antibodies can be used in methods known in the art relating to the localization and activity of the MS4A polypeptide sequences of the invention, e.g., for cloning of MS4A nucleic acids, immunopurification of MS4A polypeptides, imaging MS4A polypeptides in a biological sample, measuring levels thereof in appropriate biological samples, and in diagnostic methods.

VI. Methods for Detecting a MS4A Polypeptide

In another aspect of the invention, a method is provided for detecting a level of MS4A polypeptide using an antibody that specifically recognizes a MS4A polypeptide, or portion thereof. In a preferred embodiment, biological samples from an experimental subject and a control subject are obtained, and MS4A polypeptide is detected in each sample by immunochemical reaction with the MS4A antibody. More preferably, the antibody recognizes amino acids of any one of the even-numbered SEQ ID NOs:2-38, and is prepared according to a method of the present invention for producing such an antibody.

In one embodiment, a MS4A antibody is used to screen a biological sample for the presence of a MS4A polypeptide. A biological sample to be screened can be a biological fluid such as extracellular or intracellular fluid,

or a cell or tissue extract or homogenate. A biological sample can also be an isolated cell (e.g., in culture) or a collection of cells such as in a tissue sample or histology sample. A tissue sample can be suspended in a liquid medium or fixed onto a solid support such as a microscope slide. In accordance with a screening assay method, a biological sample is exposed to an antibody immunoreactive with a MS4A polypeptide whose presence is being assayed, and the formation of antibody-polypeptide complexes is detected. Techniques for detecting such antibody-antigen conjugates or complexes are well known in the art and include but are not limited to centrifugation, affinity chromatography and the like, and binding of a labeled secondary antibody to the antibody-candidate receptor complex.

In one embodiment, an antibody that specifically recognizes a MS4A polypeptide can be used to assess the tissue- or cell-distribution of MS4A protein, for example, to evaluate CD20 expression during B lymphocyte development (Figure 9). CD20 expression in B220⁺ lymphocytes from lymphoid tissues of wild type mice was examined by two-color immunofluorescence. In bone marrow, three types of B220⁺ cells were detected. The vast majority of B220^{hi} lymphocytes expressed CD20. However, the majority of B220^{lo} lymphocytes were CD20-negative. Thus, CD20 was predominantly expressed by mature B cells.

CD19 expression is restricted to normal and neoplastic B cells and follicular dendritic cells. CD19 is expressed early by B progenitor cells in the bone marrow, presumably at the late pro-B or early pre-B cell stages around the time of immunoglobulin heavy chain rearrangement (Anderson et al. (1984) *Blood* 63:1424). Expression persists during all stages of B cell maturation and is lost upon terminal differentiation to plasma cells.

Double staining of CD20 with IgM and CD19 antibodies showed that some of the CD19^{lo} and IgM^{lo} cells were CD20 negative in the bone marrow. A few IgM⁻ cells also expressed low levels of CD20 in the bone marrow. This data suggested that the CD20 expression was later than the CD19 expression but before or around the time of IgM expression during B cell

development in the bone marrow since these cells were gated on lymphocytes not dendritic cells.

The level of CD20 expression observed on mature B220^{hi} B cells in bone marrow was maintained by B cells from peripheral lymphoid tissues.

- 5 The vast majority of B220⁺ B cells in the spleen, blood, peripheral lymph nodes, and peritoneal cavity expressed CD20. Therefore, like human CD20, mouse CD20 was also exclusively expressed on B cells from the immature B cell stage to mature B cells.

10 VII. Identification of MS4A Modulators

VII.A. Screening for Small Molecule Ligands that Interact with a MS4A Polypeptide

- The present invention further discloses a method for identifying a compound that modulates MS4A function. According to the method, a
15 MS4A polypeptide is exposed to a plurality of compounds, and binding of a compound to the isolated MS4A polypeptide is assayed. A compound is selected that demonstrates specific binding to the isolated MS4A polypeptide. Preferably, the MS4A polypeptide used in the binding assay of the method includes some or all amino acids of any one of the even-
20 numbered SEQ ID NOs:2-38.

- Several techniques can be used to detect interactions between a protein and a chemical ligand without employing an *in vivo* ligand. Representative methods include, but are not limited to, Fluorescence Correlation Spectroscopy, Surface-Enhanced Laser Desorption/Ionization
25 Time-Of-flight Spectroscopy, and Biacore technology, as described in Example 5. These methods are amenable to automated, high-throughput screening.

- Candidate regulators include but are not limited to proteins, peptides, and chemical compounds. Structural analysis of these selectants can
30 provide information about ligand-target molecule interactions that enable the development of pharmaceuticals based on these lead structures.

Similarly, the knowledge of the structure a native MS4A polypeptide provides an approach for rational drug design. The structure of a MS4A polypeptide can be determined by X-ray crystallography or by computational algorithms that generate three-dimensional representations. See Huang et al. (2000) *Pac Symp Biocomput* 230-41; Saqi et al. (1999) *Bioinformatics* 15:521-522. Computer models can further predict binding of a protein structure to various substrate molecules, that can be synthesized and tested. Additional drug design techniques are described in U.S. Patent Nos. 5,834,228 and 5,872,011.

10 VII.B. Methods for Identifying Modulators of MS4A Gene Expression

The assembly and annotation of genomic sequences comprising MS4A genes in the region of human chromosome 11q12-13.1, disclosed herein for the first time, identify MS4A gene regulatory regions. Preferably, MS4A gene regulatory regions comprise sequences upstream of the initial coding region of each MS4A gene as disclosed in SEQ ID NOs:73-81. An expression cassette comprising a MS4A promoter region can be employed in assays for the identification of modulators of MS4A expression. Thus the present invention also provides a method for identifying a substance that regulates MS4A gene expression using a chimeric gene that includes an isolated MS4A gene promoter region operably linked to a reporter gene. According to this method, a gene expression system is established that includes the chimeric gene and components required for gene transcription and translation so that reporter gene expression is assayable. To select a substance that regulates MS4A gene expression, the method further provides the steps of using the gene expression system to determine a baseline level of reporter gene expression in the absence of a candidate regulator; providing one or more candidate regulators to the gene expression system; and assaying a level of reporter gene expression in the presence of a candidate regulator. A candidate regulator is selected whose presence results in an altered level of reporter gene expression when compared to the baseline level.

30 Several molecular cloning strategies can be used to identify

substances that specifically bind a MS4A gene cis-regulatory element. In one embodiment, a cDNA library in an expression vector, such as the lambda-gt11 vector, can be screened for cDNA clones that encode a MS4A gene regulatory element DNA-binding activity by probing the library with a
5 labeled MS4A DNA fragment, or synthetic oligonucleotide (Singh et al. (1989) *Biotechniques* 7:252-261). Preferably, the nucleotide sequence selected as a probe has already been demonstrated as a protein binding site using a protein-DNA binding assay, as described in Example 9.

In another embodiment, transcriptional regulatory proteins are
10 identified using the yeast one-hybrid system (Luo et al. (1996) *Biotechniques* 20(4):564-568; Vidal et al. (1996) *Proc Natl Acad Sci USA* 93(19):10315-10320; Li & Herskowitz (1993) *Science* 262:1870-1874). In this case, a cis-regulatory element of a MS4A gene is operably fused as an upstream activating sequence (UAS) to one, or typically more, yeast reporter genes
15 such as the *lacZ* gene, the *URA3* gene, the *LEU2* gene, the *HIS3* gene, or the *LYS2* gene, and the reporter gene fusion construct(s) is inserted into an appropriate yeast host strain. It is expected that the reporter genes are not transcriptionally active in the engineered yeast host strain, for lack of a transcriptional activator protein to bind the UAS derived from the MS4A gene
20 promoter region. The engineered yeast host strain is transformed with a library of cDNAs inserted in a yeast activation domain fusion protein expression vector, e.g. pGAD, where the coding regions of the cDNA inserts are fused to a functional yeast activation domain coding segment, such as those derived from the GAL4 or VP16 activators. Transformed yeast cells
25 that acquire a cDNA encoding a protein that binds a cis-regulatory element of a MS4A gene can be identified based on the concerted activation the reporter genes, either by genetic selection for prototrophy (e.g. *LEU2*, *HIS3*, or *LYS2* reporters) or by screening with chromogenic substrates (e.g., a *lacZ* reporter) by methods known in the art.

30 The present invention also provides an *in vivo* assay for discovery of modulators of MS4A gene expression. In this case, a transgenic non-human animal is made such that a transgene comprising a MS4A gene promoter

and a reporter gene is expressed and a level of reporter gene expression is assayable. Such transgenic animals can be used for the identification of compounds that are effective in modulating MS4A gene expression.

In vitro or *in vivo* screening approaches can also survey more than one modulatable transcriptional regulatory sequence simultaneously.

VIII. Animal Models

The present invention further pertains to an animal model of disorders associated with a MS4A nucleic acid or polypeptide, including but not limited to atopic disorders, abnormal target cell development, function, and Ca^{++} responses. Such a model can be prepared by several methods. Using a transgenic approach, knock-out, knock-in, or knock-down mutation of the MS4A gene can suppress MS4A function. The present invention also teaches that an animal model of a MS4A-related disorder can be prepared by immunizing an animal with a MS4A polypeptide. The resulting immune response in the animal comprises a production of antibodies that specifically bind a MS4A polypeptide, thereby disrupting its biological activity. A method is also provided for generating an animal model of a MS4A-related disorder by administering to an animal a compound that disrupts MS4A expression or function. Such a compound is discovered by methods disclosed herein.

VIII.A. Generation of CD20-Deficient Mice

CD20-deficient mice were generated by targeted disruption of the CD20 gene in embryonic stem (ES) cells using homologous recombination, as described in Example 6. A targeting vector was generated that replaces exons encoding part of the second extracellular loop, the 4th transmembrane domain, and the large carboxyl-terminal cytoplasmic domain of CD20 with a neomycin resistant gene (Figure 10A-D). Appropriate gene targeting generates an aberrant CD20 protein truncated at amino acid position 157 and fused with an 88 amino acid protein encoded by the *Neo^r* gene promoter sequence.

After DNA transfections, 6 of 115 Neo-resistant ES cell clones carried the targeted allele as determined by Southern blot analysis of EcoR V

digested genomic DNA using a 1.5 kb DNA probe (Figure 10D). Appropriate targeting was further verified in two clones by Southern analysis of ES cell DNA digested with BamH I (>12 kb fragment was reduced to a 6.5 kb band in targeted cells), Kpn I (7.2 kb became 5.5 kb), and Ssp I (5.6 kb became 5 7.0 kb) using the same probe. Cells of one ES cell clone were injected into blastocysts that were transferred into foster mothers. Highly chimeric male offspring (80-100% according to coat color) bred with C57BL/6 (B6) females transmitted the mutation to their progeny (Figure 10E). Mice homozygous for disruption of the *CD20* gene were obtained at the expected Mendelian 10 frequency by crossing heterozygous offspring.

Appropriate targeting of the *CD20* gene was further verified by PCR analysis of genomic DNA from homozygous offspring (Figure 10F). Wild type *CD20* mRNA was absent in *CD20*^{-/-} mice as confirmed by PCR amplification of cDNA generated from splenocytes of *CD20*^{-/-} mice (Figure 15 10G). *CD20*-deficient mice (*CD20*^{-/-}) thrived and reproduced as well as their wild type littermates and did not present any obvious anatomical or morphological abnormalities during the first year of life.

Absence of cell surface *CD20* protein expression in *CD20*^{-/-} mice was further verified by staining B220⁺ splenocytes with murine anti-*CD20* 20 monoclonal antibodies. Hybridomas producing these antibodies were generated using splenocytes from *CD20*^{-/-} mice that were immunized with *CD20*-GFP cDNA-transfected 300.19 cells. Ten hybridomas secreted antibodies reactive with 300.19 (Figure 10H) and CHO (Figure 10I) cells transfected with *CD20*-GFP cDNA, but not with untransfected CHO or 25 300.19 cells (Table 6). These antibodies also reacted with *CD20* epitopes expressed on the cell surface of B220⁺ splenocytes from wild type mice, but not with splenocytes from *CD20*^{-/-} mice (Figure 10J). Therefore, targeted mutation of the *CD20* gene abrogated cell surface *CD20* protein expression.

Table 6
Anti-CD20 Monoclonal Antibodies Generated in CD20^{-/-} Mice^a

Ab Name	Clone Name	Isotype	Whole Cell ELISA ^a		FACS Analysis ^b		
			CD20-CHO	CHO	CD20-300.19	300.19	Spleen
MB20-1	MCD20-5	IgG1, K	+	-	+	-	+
MB20-2	MCD20-61	IgG1, K	+	-	+	-	++
MB20-3	MCD20-86	IgG3, K	+	-	+	-	++
MB20-6	MCD20-223	IgG2a, K	+	-	+	-	+
MB20-7	MCD20-243	IgG2b, K	+	-	+	-	+
MB20-8	MCD20-270	IgG2b, K	+	-	+	-	+
MB20-10	MCD20-388	IgG2b, K	+	-	+	-	+
MB20-11	MCD20-392	IgG2a, K	+	-	+	-	+
MB20-13	MCD20-624	IgG3, K	+	-	+	-	++
MB20-14	MCD20-642	IgG1, K	+	-	+	-	++

^aValues represent reactivity of the monoclonal antibody with adherent
5 monolayers of CHO cells either transfected or untransfected with CD20-GFP
cDNA as assessed by a cell-based ELISA. The monoclonal antibodies did
not react with GFP cDNA-transfected CHO cells.

^bCell surface reactivity of the monoclonal antibody with single cell
suspensions of 300.19 cells either transfected or untransfected with CD20-
10 GFP cDNA or spleen cells from wild type mice. Values represent relative
indirect immunofluorescence staining intensity as assessed by flow
cytometry and shown in figure 10H-J.

VIII.B. B Cell Development and Function in CD20^{-/-} Mice

CD20^{-/-} mice did not show an obvious propensity for infections during their first year of life. They had normal frequencies of IgM⁻ B220^{lo} pro/pre-B cells, IgM⁺ B220^{lo} immature B cells and IgM⁺ B220^{hi} mature B cells in the bone marrow (Figure 11, Table 7). Overall, the number of circulating and spleen IgM⁺ B220⁺ B cells found in CD20^{-/-} mice was increased compared with wild type littermates (Table 7). However, an immunohistochemical analysis of spleen tissue sections revealed a normal architecture and organization of the spleen. In the bone marrow, overall IgM expression was decreased on immature B cells, yet increased on mature B cells when compared with IgM levels expressed by comparable cells in wild type littermates. However, overall IgM expression by mature B220^{hi} B cells in the blood, spleen and lymph nodes was slightly lower in CD20^{-/-} mice (Figure 11B-D). There were no obvious differences in the size (light scatter properties) of CD20^{-/-} B cells isolated from bone marrow, blood, lymph nodes or spleen when compared with B cells from wild type littermates. These data therefore suggest that CD20 plays a functional role in the development and tissue localization of B cells.

Table 7

Frequencies and Numbers of B Lymphocytes in CD20^{-/-} Mice

Tissue	Phenotype	Wild Type	CD20 ^{-/-}	Wild Type	CD20 ^{-/-}
		% of B Lymphocytes		B cell numbers (x10 ⁻⁶)	
Bone Marrow	B220 ^{lo} IgM ⁺	36 ± 2	34 ± 3		
	B220 ^{lo} IgM ⁺	19 ± 2	13 ± 2*		
	B220 ^{hi} IgM ⁺	14 ± 2	16 ± 4		
Blood ^d	B220 ⁺ IgM ⁺	61 ± 2	60 ± 3	3.6 ± 0.5	3.9 ± 0.5
Spleen	B220 ⁺ IgM ⁺	51 ± 6	53 ± 5	58 ± 12	76 ± 12
Lymph Nodes ^e	B220 ⁺ IgM ⁺	26 ± 6	19 ± 2	1.2 ± 0.3	0.9 ± 0.3
Peritoneum	B220 ⁺ IgM ⁺	70 ± 4	69 ± 5	2.4 ± 0.3	3.1 ± 0.3
	B220 ^{lo} CD5 ⁺	44 ± 4	15 ± 5**	1.5 ± 0.2	0.7 ± 0.2
	B220 ^{hi} CD5 ⁺	28 ± 2	59 ± 3**	1.0 ± 0.1	2.7 ± 0.1

5 ^aValues represent mean (± SEM) results obtained from seven 2-month-old of wild type controls and 10 CD20^{-/-} mice. Numbers represent the percentage of lymphocytes (based on side and forward light scatter properties) expressing the indicated cell surface markers.

^bB cell numbers were calculated based on the total number of cells harvested from the indicated tissues.

10 ^dThe values indicate the number of cells/ml.

^eValues represent results from peripheral lymph nodes pairs.

*The percentage or number was significantly different than in wild-type, $p < 0.05$; ** $p < 0.01$.

Within the peritoneal cavity, the number of IgM⁺ B220⁺ B cells in CD20^{-/-} mice was similar to that of wild-type littermates (Table 7, Figure 11E). However, there was a 4-fold decrease in the number of CD5⁺ B220^{lo} B1a cells, with a compensatory increase in the number of CD5⁻ B220^{hi} B2 cells. Therefore, CD20-deficiency predominantly affected the development or clonal expansion of the B1 subpopulation of B cells within the peritoneal cavity. Exemplary methods for quantitating B cell populations are described in Example 7.

VIII.C. Reduced [Ca⁺⁺]_i Responses in CD20^{-/-} B Cells

10 The loss of CD20 significantly altered early B cell signaling responses, measured as described in Example 8. Splenic B220⁺ B cells from CD20^{-/-} mice generated substantially reduced [Ca⁺⁺]_i responses following surface IgM ligation when compared with wild type B cells. Decreased [Ca⁺⁺]_i responses in CD20^{-/-} B cells were observed in response to both optimal (40 µg/ml, Figure 12A) and suboptimal concentrations (5 µg/ml) of anti-IgM antibodies. Although the kinetics of [Ca⁺⁺]_i responses in CD20^{-/-} B cells was not altered, the magnitude of both the immediate [Ca⁺⁺]_i increase and the sustained increase observed at later time points were inhibited by loss of CD20 expression. More dramatic decreases in [Ca⁺⁺]_i responses (>50%) by CD20^{-/-} B cells were observed in response to CD19 ligation with optimal concentrations (40 µg/ml) of antibody (Figure 12A). Reduced [Ca⁺⁺]_i responses following CD19 ligation on CD20^{-/-} B cells were likely to result from differences in signaling capacity since Thapsigargin-induced (Figure 12A) and Ionomycin-induced [Ca⁺⁺]_i responses were higher in CD20^{-/-} B cells than in wild type B cells. In addition, CD19 expression levels were not significantly different between CD20^{-/-} and wild type B cells (Figure 12A).

Chelation of extracellular calcium with EGTA reduced the kinetics and magnitude of the immediate [Ca⁺⁺]_i increase observed following IgM

crosslinking (Figure 12A). However, the $[Ca^{++}]_i$ increase observed at later time points was not substantially inhibited by EGTA treatment. Similar results were observed in CD20^{-/-} B cells. By contrast, chelation of extracellular calcium with EGTA almost eliminated the $[Ca^{++}]_i$ response observed following CD19 crosslinking (Figure 12A). This suggests that transmembrane Ca^{++} flux contributes substantially to the $[Ca^{++}]_i$ responses observed following CD19 crosslinking. That CD20-deficiency had a substantial effect on CD19-induced $[Ca^{++}]_i$ responses suggests that CD20 can contribute significantly to transmembrane Ca^{++} flux.

10 The consequences of CD20 loss on transmembrane signal transduction was further evaluated by assessing total cellular protein tyrosine phosphorylation in purified B cells following IgM ligation. Although some variation was observed between B cells from individual mice in individual experiments, overall levels of tyrosine phosphorylation in resting splenic B cells were higher in CD20^{-/-} B cells than in wild type mice (Figure 12C). In addition, protein phosphorylation in B cells from CD20^{-/-} mice increased more significantly after B cell antigen receptor (BCR) ligation than in wild type B cells. Thus, while CD20 expression can influence BCR-induced tyrosine phosphorylation, decreased $[Ca^{++}]_i$ responses in CD20^{-/-} B cells are unlikely to result from significant abnormalities in transmembrane signaling through the BCR.

IX. Therapeutic Applications

25 Another aspect of the present invention is a therapeutic method comprising administering to a subject a substance that modulates MS4A biological activity. Therapeutic substances include but are not limited to chemical compounds, antibodies, and gene therapy vectors. Substances that are discovered by the methods disclosed herein are useful for therapeutic applications related to disorders of MS4A function.

In one embodiment, the present invention provides a method for disrupting MS4A function by immunizing a subject with an effective dose of the disclosed MS4A polypeptide. The immune system of the subject produces an antibody that specifically recognizes the MS4A polypeptide, and binding of the antibody to the MS4A polypeptide abolishes MS4A function.

In another embodiment, the present invention provides MS4A nucleic acid sequences and gene therapy methods for modulating MS4A activity in a target cell. The gene therapy vector can encode a MS4A or sequences encoding a nucleic acid molecule, peptide, or protein that interacts with a MS4A protein.

Vehicles for delivery of a gene therapy vector include but are not limited to a liposome, a cell, and a virus. Preferably, a cell is transformed or transfected with the DNA molecule or is derived from such a transformed or transfected cell. Alternatively, the vehicle is a virus, including a retroviral vector, adenoviral vector or vaccinia virus whose genome has been manipulated in alternative ways so as to render the virus non-pathogenic. Methods for creating such a viral mutation are detailed in U.S. Patent No. 4,769,331. Exemplary gene therapy methods are also described in U.S. Patent Nos. 5,279,833; 5,286,634; 5,399,346; 5,646,008; 5,651,964; 5,641,484; and 5,643,567.

The therapeutic methods of the present invention can be applied in the treatment of a variety of conditions, including in the treatment of non-Hodgkin's lymphoma and in the treatment of atopic disorders or other allergic diseases. Application of the present inventive therapeutic methods are evidenced by the current U.S. Food and Drug Administration approved use of antibodies against CD20 in the treatment of non-Hodgkin's lymphoma. Additionally, the therapeutic methods of the present invention are illustrated in view of the recognition in the art that genetic variations at chromosome 11Q12-13 can also play a role in the pathogenesis of atopic disorders and other allergic diseases. Indeed, it has been recognized that

FcεRIβ contributes to such diseases, and thus the MS4A genes identified in accordance with the present invention are envisioned also to contribute to allergenic disease. Therefore the present therapeutic methods, which pertain to the modulation of the biological activity of an MS4A polypeptide of the present invention have application with respect to the treatment of such disorders.

X. Summary

The invention comprises 19 new genes that are members of a class of genes encoding MS4A proteins. Three members have been described, CD20, FcεRIβ, and HTm4. A gene family has been defined based on a shared chromosomal location, conservation of protein size and structure, gene structure conservation, and similar expression in hematopoietic cells. MS4A proteins function as oligomeric cell surface complexes, and complex assembly using diverse MS4A members is implicated as a mechanism for regulating complex function.

Two members of this class, CD20 and FcεRIβ, have been described functionally, and in each case an important function has been delineated. CD20 is required for cell cycle progression and signal transduction in B lymphocytes. CD20 also regulates Ca⁺⁺ conductance, possibly as a cation channel subunit. Of clinical relevance, antibodies that recognize CD20 are effective in treating non-Hodgkin's lymphoma. FcεRIβ mediates interactions with IgE-bound antigens that lead to degranulation of mast cells, and variation of the FcεRIβ locus is implicated in allergenic disease.

The utility of the MS4A genes is based in part on overlapping or shared functions with known MS4A members. In one case, new MS4A genes have important potential as part of a CD20 complex. The structural description of CD20 complexes suggests that one or more CD20-related proteins constitute the functional complex. Thus, new MS4A proteins can define antigens useful for lymphoma treatment. In another case, MS4A

genes are implicated in IgE responses. Atopic disorders (allergy, asthma, eczema, allergic rhinitis) are dysfunctional IgE responses and are associated with a locus on human chromosome 11q containing most members of the MS4A gene family. FcεRIβ is one relevant factor, and recent work supports
5 that FcεRIβ as well as other genetic elements in the region contribute to the disease. Thus, as disclosed herein, the present MS4A sequences also have utility in the characterization, diagnosis, and potential treatment of atopy linked to the chromosomal location wherein MS4A genes are located.

10 Examples

The following Examples have been included to illustrate modes of the invention. Certain aspects of the following Examples are described in terms of techniques and procedures found or contemplated by the present co-inventors to work well in the practice of the invention. These Examples
15 illustrate standard laboratory practices of the co-inventors. In light of the present disclosure and the general level of skill in the art, those of skill will appreciate that the following Examples are intended to be exemplary only and that numerous changes, modifications, and alterations can be employed without departing from the scope of the invention.

20 Example 1

Database Searches and cDNA Isolation

Three hundred and thirty seven nucleotide sequences obtained from the translated GenBank database of expressed sequence tags (ESTs) were assembled into sixty-two subgroups of contiguous linear segments based on
25 their overlapping sequences and potential for encoding proteins homologous with CD20. Based on these subgroups, EST cDNAs (Figure 1) were obtained from the ATCC and sequenced. Based on the complete sequences of twenty-one near full-length EST cDNAs, eleven novel genes were defined in human and mouse that unified multiple EST subgroups.
30 Near full-length EST clones representing these genes are shown in Figure 1.

These eleven genes and five additional genes were also identified by PCR amplification of transcripts using subgroup-specific primers or primers based on EST sequences. The specific details of how cDNAs representing the five genes that were not identified by EST cDNA clones are indicated below. In all cases, ESTs and cDNAs encoding the predicted coding regions of each putative unique gene were sequenced in both directions and at least two independent ESTs and/or cDNAs representing near full-length gene products were sequenced. Thereby, there was independent confirmation of accuracy for all of the sequences reported.

Based on EST subgroup sequences, cDNAs encoding mouse *MS4a4B* and *MS4a4C* were isolated by PCR amplification of C57BL/6 mouse spleen cDNA using both Taq and Pfu DNA polymerase. Primers for *MS4a4B* (SEQ ID NOs:63-64) amplified an 879 bp fragment. Primers for *MS4a4C* (SEQ ID NOs:65-66) amplified a 794 bp fragment. EST sequences for *MS4a4D* only encoded the 3' end of the predicted protein. Since *MS4a4D* sequences were closely related to *MS4a4B* and *MS4a4C* sequences, a sense 5' primer (SEQ ID NO:67) based on consensus *MS4a4B* and *MS4a4C* sequences and a *MS4a4D*-specific antisense primer (SEQ ID NO:68) were used to amplify a 773 bp fragment from cDNA of C57BL/6 mouse lung.

MS4a6C was initially identified based on one unique EST sequence (AA028258) encoding a mouse protein homologous with the C-terminal end of *MS4a6B*. *MS4a6C* cDNAs were isolated by PCR amplification of C57BL/6 mouse bone marrow cDNA using Taq polymerase. A primer based on identical sequences at the 5' end of the *MS4a6B* and *MS4a6D* cDNAs (SEQ ID NO:69) was used in combination with an antisense primer specific for the unique EST sequence (SEQ ID NO:70) to amplify a 787 bp fragment. Sequences from multiple independent PCR-amplified cDNAs were identical. Subsequently, the PCR-generated 5' end of the near full-length *MS4a6C* cDNA was found to be identical to an orphan EST subgroup sequence that had not been linked with defined 3' sequences. Thereby, the EST subgroup sequences verified that the PCR-amplified 5' end of the *MS4a6C* cDNAs

was appropriate. In addition, the overall *MS4a6C* sequence was similar to the sequence of *MS4a6B* cDNAs without interruption. Thus, the *MS4a6C* cDNA united sequences identical to those found in two non-overlapping CD20-homologous EST subgroups. cDNAs encoding a 473 bp fragment of mouse *MS4a3* were amplified from cDNA of C57BL/6 bone marrow as described above. Primers (SEQ ID NOs:71-72) were obtained based on a single thymic cDNA EST sequence (GenBank AA940479) where the corresponding cDNA was not available.

Human MS4A and mouse MS4a cDNA sequences (MS4A1 to MS4A12) (disclosed herein) were used to search the htgs GenBank human genomic database of unfinished human genomic sequences (<http://www.ncbi.nlm.nih.gov/blast/>) using the BLAST program. Seventeen phase 1 or phase 2 human genomic DNA sequences encoding potential MS4A genes were assembled into groups of contiguous linear segments based on their overlapping sequences. Three EST clones corresponding to partial *MS4A6E* transcripts were obtained from the ATCC and sequenced completely on both DNA stands.

All PCR-amplified cDNAs were subcloned and sequenced entirely in both directions. Complete sequencing of at least two distinct PCR-generated cDNAs from both Taq and Pfu enzyme was performed in most cases. Differences between cDNA sequences were only noted when multiple cDNA clones generated by both Taq and Pfu polymerases revealed identical differences. In some cases, cDNAs or EST sequences contained potential intron|exon splice sites that delimited structural domains and aligned with the known intron|exon splice sites of CD20 (Tedder et al. (1989b) *J Immunol* 142:2560-2568). In these cases, potential introns were flanked by consensus splice donor and/or splice acceptor sequences (Aebi & Weissmann (1987) *Trends Genet* 3:102-107) or were likely to represent splice variants where exons were deleted.

Example 2

RNA Isolation and Reverse Transcription-PCR

Reverse transcription-PCR amplification (RT-PCR) was as described previously (Zhou & Tedder, 1995) with minor modifications. Total RNA was
5 extracted from $1-2 \times 10^7$ hematopoietic cell lines using a RNeasy Mini Kit (Qiagen, Inc., Chatsworth, California) according to the manufacturer's instructions. Human hematopoietic cell lines included one pre-B cell line (NALM-6), three B cell lines (BJAB, DAUDI, and SB), four T cell lines (HSB-2, HUT-78, JURKAT, and MOLT15), two myelomonocytic lines (HL60 and
10 U937), and one erythroleukemia cell line (K562). RNA concentrations were determined by UV absorbance. Ten μg of total RNA was reverse transcribed. In some cases, cDNA from any of 8 different human tissues (colon, ovary, blood mononuclear cells, prostate, small intestine, spleen, testes, and thymus; from CLONETECH Laboratories, Inc., Palo Alto,
15 California) was analyzed. RT-PCR amplification was performed using gene-specific primers identical with protein coding regions of the predicted MS4A genes during 35 cycles (94°C for 1 min, 55°C for 1.5 min, 72°C for 1.5 min, followed by extension at 72°C for 5 min). Following amplification, the PCR products were separated on 1% agarose-ethidium bromide gels and
20 photographed. G3PDH, a housekeeping gene, was also amplified to control for sample to sample variation. RNA amplified without reverse transcription was used as a negative control, and was negative in all cases.

Example 3

Recombinant Production of MS4A Protein

25 For recombinant production of a protein of the invention in a host organism, a nucleotide sequence encoding the protein is inserted into an expression cassette designed for the chosen host and introduced into the host where it is recombinantly produced. The choice of the specific regulatory sequences such as promoter, signal sequence, 5' and 3'
30 untranslated sequence, and enhancer appropriate for the chosen host is within the level of ordinary skill in the art. The resultant molecule, containing

the individual elements linking in the proper reading frame, is inserted into a vector capable of being transformed into the host cell. Suitable expression vectors and methods for recombinant production of proteins are well known for host organisms such as *E. coli*, yeast, and insect cells (see, e.g.,
5 Lucknow & Summers (1988) *Bio/Technol* 6:47). Additional suitable expression vectors are baculovirus expression vectors, e.g., those derived from the genome of *Autographica californica* nuclear polyhedrosis virus (AcMNPV).

Recombinantly produced proteins are isolated and purified using a
10 variety of standard techniques. The actual techniques used varies depending upon the host organism used, whether the protein is designed for secretion, and other such factors. Such techniques are well known to the skilled artisan. See Ausubel et al. (1994).

Example 4

15 Mouse Anti-Mouse CD20 Monoclonal Antibody Production

Hybridomas producing CD20-specific mouse monoclonal antibodies were generated by the fusion of NS-1 myeloma cells with spleen cells from a CD20^{-/-} mouse immunized with a cell line expressing a mouse CD20-GFP fusion protein. The CD20-GFP fusion protein was generated by subcloning
20 a fragment of the pmB1-1 cDNA (from 159 to 1050 bp of SEQ ID NO:39) into the PEGFP-N1 vector (Clontech Laboratories Inc., Palo Alto, California) to generate an open reading frame encoding the entire CD20 protein with GFP fused to the carboxyl-terminal end. The resulting plasmid was linearized with ApaL I and used to transfect 300.19 cells, a mouse pre-B cell line, and
25 Chinese Hamster Ovary (CHO) cells. Transfection was by Lipofectamine following the manufacturer's instructions (Clontech Laboratories, Inc.). Transfected cells were selected using GENETICIN™ (1 mg/ml, GIBCOBRL) in RPMI 1640 media (Sigma) for 300.19 cells or H-12 nutrient mixture (GIBCOBRL) for CHO cells. Both media were supplemented with 10% FCS,
30 L-glutamine, streptomycin and penicillin. Transfected cells expressing high levels of CD20-GFP were isolated by fluorescence-based cell sorting.

Example 5

In vitro Binding Assays

Recombinant protein can be obtained, for example, according to the approach described in Example 4 herein above. The protein is immobilized
5 on chips appropriate for ligand binding assays. The protein immobilized on the chip is exposed to sample compound in solution according to methods well known in the art. While the sample compound is in contact with the immobilized protein, measurements capable of detecting protein-ligand interactions are conducted. Measurement techniques include, but are not
10 limited to, SEDLI, Biacore, and FCS, as described above. Compounds found to bind the protein are readily discovered in this approach and are subjected to further characterization.

Example 6

Generation of CD20-Deficient Mice

15 DNA encoding the *CD20* gene was isolated from a phage library prepared from 129/Sv strain mouse DNA (Figure 10A), mapped with restriction endonucleases, and sequenced to identify intron|exon boundaries (Figure 10B). The targeting vector was constructed using a pBluescript SK (Stratagene, La Jolla, California)-based targeting vector (p594, provided by
20 Dr. David Milstone, Brigham and Women's Hospital, Boston, Massachusetts). A DNA fragment starting at the Pst I site in *CD20* exon 5 through the EcoR V site in exon 6 (~1.8 kb) was isolated and blunt end ligated into the targeting vector downstream of the pMC1-HSV thymidine kinase gene and upstream of the neomycin resistance marker obtained from
25 pGK-neo poly A (Stratagene) that contained the PGK promoter and poly A signal sequence. An ~10 kb DNA fragment beginning at the Kpn I site downstream of exon 8 was also isolated and inserted into the targeting vector downstream of the neomycin resistant gene. The plasmid was linearized using a unique Sal I restriction site proximal to the 3' end of the
30 *CD20* gene insert and used to transfect ES cells.

ES cells were transfected with linearized plasmid DNA and selected for G418 resistance as described (Keller and Smithies (1989) *Proc Natl Acad Sci USA* 86:8932). Genomic DNA from individual selected clones was digested with EcoR V and used for Southern blot analysis along with a radiolabeled ~1.5 kb DNA probe that was external to the targeting vector (Figure 10D). A 4.6 kb genomic DNA fragment hybridized with the probe in wild type ES cells or a 6.3 kb fragment in appropriately targeted ES cells (Figure 1E). Genomic DNA generated by BamH I, Ssc I or Kpn I digestion was also analyzed for appropriate targeting. The Southern blot pattern obtained in all cases was consistent with the appropriate predicted mutation indicating that detrimental recombinations did not occur in the vicinity of the desired homologous recombination. Cells from appropriately targeted ES cell clones were injected into 3.5 day old C57BL/6 blastocysts that were transferred into foster mothers. Offspring carrying the mutant CD20 allele were identified by Southern blot analysis of DNA obtained from tail biopsies.

High chimeric males (80-100% according to color) were bred with C57BL/6 (B6) females to generate heterozygous offspring with germline gene transmission, which were crossed to generate the homozygous CD20^{-/-} and wild type littermates used for this study. In some cases, B6/129F1J (Jackson Laboratory) were used as controls. Results obtained using wild type littermates of CD20^{+/-} mice were similar and were therefore pooled. All mice were between 2-3 months of age when used for this study. Mice were housed in a specific pathogen-free barrier-facility. All studies and procedures were approved by the Animal Care and Use Committee of Duke University.

Example 7

Flow Cytometric Analysis of Lymphocyte Subsets

Single cell suspensions of lymphocytes from the spleen, bone marrow, peripheral lymph nodes, and peritoneal cavity were isolated from CD20^{-/-} and wild type mice and counted using a hemocytometer prior to two-color immunofluorescence analysis. Retroorbital venous plexus puncture

was utilized to obtain circulating leukocytes. Leukocytes (0.5×10^6) were stained at 4°C using predetermined optimal concentrations of the test monoclonal antibody for 20 min as described (Zhou et al. (1994) *Mol Cell Biol* 14:3884-3894). Blood erythrocytes were lysed after staining using the
5 Coulter Whole Blood Immuno-Lyse kit as detailed by the manufacturer (Coulter, Inc., Miami, Florida). Cells were washed and analyzed on a FACScan flow cytometer (Becton Dickinson, San Jose, California).

Antibodies used in this study included the following: biotin, FITC-conjugated anti-B220 Mab (CD45RA, RA-3, 6B2, provided by Dr. Robert
10 Coffman, DNAXCORP, Palo, Alto, California); PE-conjugated anti-mouse Thy1.2 (Caltag Laboratories, Burlingame, California); B220-PE (Caltag Laboratories, Burlingame, California); biotin-conjugated anti-I-A (BD PharMingen, Franklin Lakes, New Jersey); PE or APC-conjugated anti-CD5 (BD PharMingen); PE-conjugated goat anti-mouse IgG3-specific antibody
15 (Southern Biotechnology Associates Inc., Birmingham, Alabama); and biotin-conjugated anti-mouse IgD (Southern Biotechnology Associates Inc., Birmingham, Alabama). FITC or biotin-conjugated goat anti-mouse IgM isotype-specific antibodies (Southern Biotechnology Associates Inc., Birmingham, Alabama) were also used.

20 Phycoerythrin-conjugated Streptavidin (Southern Biotechnology Associates Inc., Birmingham, Alabama) was used to reveal biotin-coupled monoclonal antibody staining. The percent positively stained lymphocytes was determined using a FACScan flow cytometer (Becton Dickinson, San Jose, California). Positive and negative populations of cells were
25 determined by using unreactive monoclonal antibody (Caltag Laboratories, Burlingame, California) as controls for background staining. Background levels of staining were delineated using gates positioned to include 98% of the control cells. Ten thousand cells with the forward and side light scatter properties of lymphocytes were analyzed for each sample.

Example 8

Intracellular Calcium Measurements

Changes in lymphocyte $[Ca^{2+}]_i$ levels were monitored by flow cytometry analysis as described (Fujimoto et al. (1999) *Immunity* 11:191).

5 Single cell suspension of splenocytes were resuspended (1×10^7 /ml) in RPMI 1640 medium containing 5% FBS, 10 mM HEPES and loaded with $1\mu M$ of indo-1-AM for 30 min at $37^\circ C$. Splenocytes were then washed and incubated with a predetermined optimal concentration of FITC-conjugated anti-B220 monoclonal antibody for 15 min at room temperature. The

10 splenocytes were washed again and resuspended at 2×10^6 /ml in medium. The fluorescence ratio (405/525 nm) of B220⁺ splenic B cells was monitored by flow cytometry at baseline for 1 min and for 6 min after stimulation with optimal and suboptimal concentrations of goat F(ab')₂ anti-IgM antibody (5-40 μg /ml), optimal concentrations of anti-mouse CD19 monoclonal antibody

15 (40 μg /ml), Thapsigargin (1 μg /ml; Sigma), or Ionomycin (2.67 μg /ml; Calbiochem Biosciences, Inc., La Jolla, California). In some cases, EGTA (5 mM final; pH 7.0) was added to the cells, immediately followed by stimulation with the inducing agents described above. Results were plotted as the fluorescence ratio at 20 sec intervals with background fluorescence

20 subtracted. An increase in the fluorescence ratio indicates an increase in $[Ca^{2+}]_i$.

Example 9

Characterization of a MS4A Promoter Region

A preferred *in vitro* technique for evaluating MS4A promoter function

25 is a transient transfection assay. According to this method, one or more chimeric reporter genes comprising a MS4A promoter region is introduced into a relevant host cell (e.g., a hematopoietic cell), and the resulting level of reporter gene expression is quantitated. Representative methods for making an expression system comprising a promoter region operably linked to a

30 heterologous reporter sequence are disclosed in U.S. Patent No. 6,087,111.

To analyze the function of a MS4A promoter region *in vivo*, transgenic

mice bearing a chimeric gene comprising a MS4A promoter region are generated, and a level of reporter gene expression in each mouse is determined.

Within a candidate promoter region or response element, the
5 presence of regulatory proteins bound to a nucleic acid sequence can be detected using a variety of methods well known to those skilled in the art (Ausubel et al., 1992). Briefly, *in vivo* footprinting assays demonstrate protection of DNA sequences from chemical and enzymatic modification within living or permeabilized cells. Similarly, *in vitro* footprinting assays
10 show protection of DNA sequences from chemical or enzymatic modification using protein extracts. Nitrocellulose filter-binding assays and gel electrophoresis mobility shift assays (EMSAs) track the presence of radiolabeled regulatory DNA elements based on provision of candidate transcription factors. Computer analysis programs, for example TFSEARCH
15 version 1.3 (Yutaka Akiyama: "TFSEARCH: Searching Transcription Factor Binding Sites", <http://www.rwcp.or.jp/papia/>), can also be used to locate consensus sequences of known cis-regulatory elements within a genomic region.

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The publications and other materials listed below and/or set forth in the text above to illuminate the background of the invention, and in particular cases, to provide additional details respecting the practice, are incorporated
5 herein by reference. Materials used herein include but are not limited to the following listed references.

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It will be understood that various details of the invention can be changed without departing from the scope of the invention. Furthermore, the foregoing description is for the purpose of illustration only, and not for the

20 purpose of limitation--the invention being defined by the claims.

CLAIMS

What is claimed is:

1. An isolated MS4A polypeptide, or functional portion thereof, comprising:
 - 5 (a) a polypeptide encoded by the nucleotide sequence of any one of the odd-numbered SEQ ID NOs:1-37;
 - (b) a polypeptide encoded by a nucleic acid molecule that is substantially identical to any one of the odd-numbered SEQ ID NOs:1-37;
 - 10 (c) a polypeptide having the amino acid sequence of any one of the even-numbered SEQ ID NOs:2-38;
 - (d) a polypeptide that is a biological equivalent of the polypeptide of any one of the even-numbered SEQ ID NOs:2-38; or
 - 15 (e) a polypeptide which is immunologically cross-reactive with an antibody that shows specific binding with a polypeptide of any one of the even-numbered SEQ ID NOs:2-38.
2. An isolated nucleic acid molecule encoding a MS4A
20 polypeptide, comprising:
 - (a) the nucleotide sequence of any one of the odd-numbered SEQ ID NOs:1-37; or
 - (b) a nucleic acid molecule substantially identical to any one of the odd-numbered SEQ ID NOs:1-37.
- 25 3. The isolated nucleic acid molecule of claim 2, comprising a 20 nucleotide sequence that is identical to a contiguous 20 nucleotide sequence of any one of the odd-numbered SEQ ID NOs:1-37.
4. A chimeric gene, comprising the nucleic acid molecule of claim 2 operably linked to a heterologous promoter.

5. A vector comprising the chimeric gene of claim 4.
6. A host cell comprising the chimeric gene of claim 4.
7. The host cell of claim 6, wherein the cell is selected from the group consisting of a bacterial cell, a hamster cell, a mouse cell, and a
5 human cell.
8. A method of detecting a nucleic acid molecule that encodes a MS4A polypeptide, the method comprising:
 - (a) procuring a biological sample comprising nucleic acid material;
 - 10 (b) hybridizing the nucleic acid molecule of claim 2 under stringent hybridization conditions to the biological sample of (a), thereby forming a duplex structure between the nucleic acid of claim 2 and a nucleic acid within the biological sample; and
 - 15 (c) detecting the duplex structure of (b), whereby a MS4A nucleic acid molecule is detected.
9. An antibody that specifically recognizes a MS4A polypeptide of claim 1.
10. A method for producing an antibody that specifically
20 recognizes a MS4A polypeptide, the method comprising:
 - (a) recombinantly or synthetically producing a MS4A polypeptide, or portion thereof;
 - (b) formulating the polypeptide of (a) whereby it is an effective immunogen;
 - 25 (c) administering to an animal the formulation of (b) to generate an immune response in the animal comprising production of antibodies, wherein antibodies are present in the blood serum of the animal; and

- (d) collecting the blood serum from the animal of (c), the blood serum comprising antibodies that specifically recognize a MS4A polypeptide.

11. A method for detecting a level of MS4A polypeptide, the
5 method comprising

- (a) obtaining a biological sample comprising peptidic material; and
- (b) detecting a MS4A polypeptide in the biological sample of (a) by immunochemical reaction with the antibody of
10 claim 9, whereby an amount of MS4A polypeptide in a sample is determined.

12. A method for identifying a substance that modulates MS4A function, the method comprising:

- (a) isolating a MS4A polypeptide of claim 1;
- 15 (b) exposing the isolated MS4A polypeptide to a plurality of substances;
- (c) assaying binding of a substance to the isolated MS4A polypeptide; and
- (d) selecting a substance that demonstrates specific
20 binding to the isolated MS4A polypeptide.

13. A method for modulating MS4A function in a subject, the method comprising:

- (a) preparing a pharmaceutical composition, comprising a substance identified according to the method of claim 10 or 12, and a carrier; and
- 25 (b) administering an effective dose of the pharmaceutical composition to a subject, whereby MS4A activity is altered in the subject.

14. The method of claim 13, wherein the substance is an antibody, a protein, a peptide, or a chemical compound.

15. The method of claim 13, wherein MS4A activity is regulation of the abundance of target cell subpopulations.

5 16. The method of claim 13, wherein MS4A activity is regulation of $[Ca^{2+}]_i$ levels.

17. A method for identifying a candidate compound as a modulator of MS4A gene expression, the method comprising:

10 (a) exposing a cell sample with a candidate compound to be tested, the cell sample containing at least one cell containing a DNA construct comprising a modulatable transcriptional regulatory sequence of a MS4A-encoding nucleic acid and a reporter gene which is capable of producing a detectable signal;

15 (b) evaluating an amount of signal produced in relation to a control sample; and

(c) identifying a candidate compound as a modulator of MS4A gene expression based on the amount of signal produced in relation to a control sample.

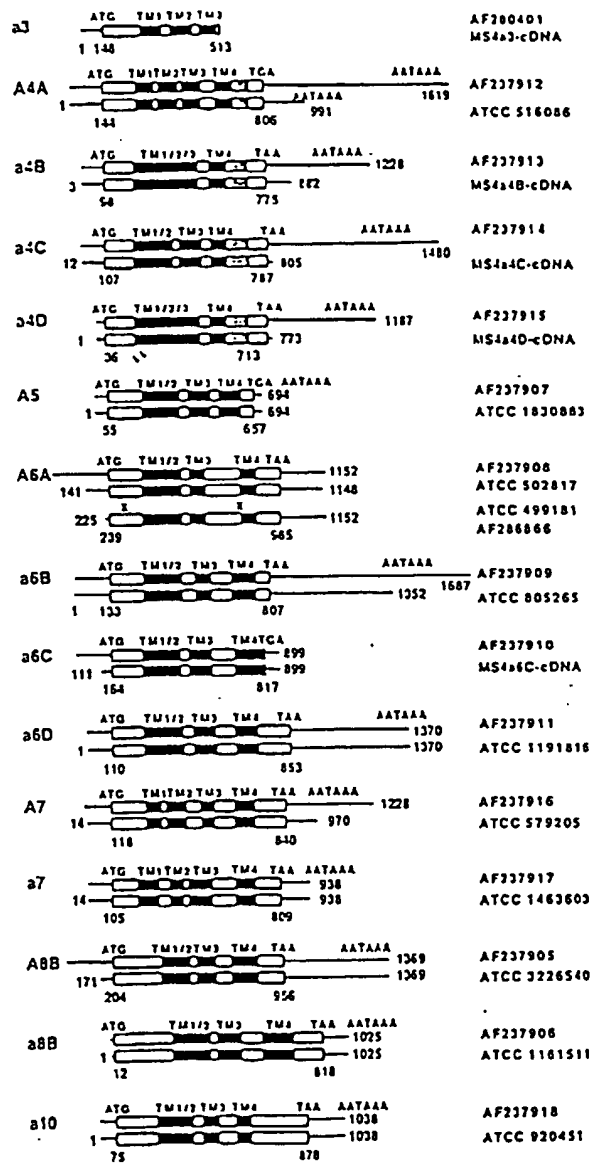
20 18. The method of claim 17, wherein the modulatable transcriptional regulatory sequence of a MS4A-encoding nucleic acid comprises a sequence that is immediately upstream of the initial coding region of a MS4A gene as set forth in any one of SEQ ID NOs:73-81.

25 19. A method for modulating MS4A function in a subject, the method comprising:

30 (a) preparing a gene therapy vector having a nucleotide sequence encoding a MS4A polypeptide or a nucleotide sequence encoding a nucleic acid molecule, peptide, or protein that interacts with a MS4A nucleic acid or polypeptide; and

- (b) administering the gene therapy vector to a subject, whereby the function of MS4A in the subject is modulated.

Figure 1



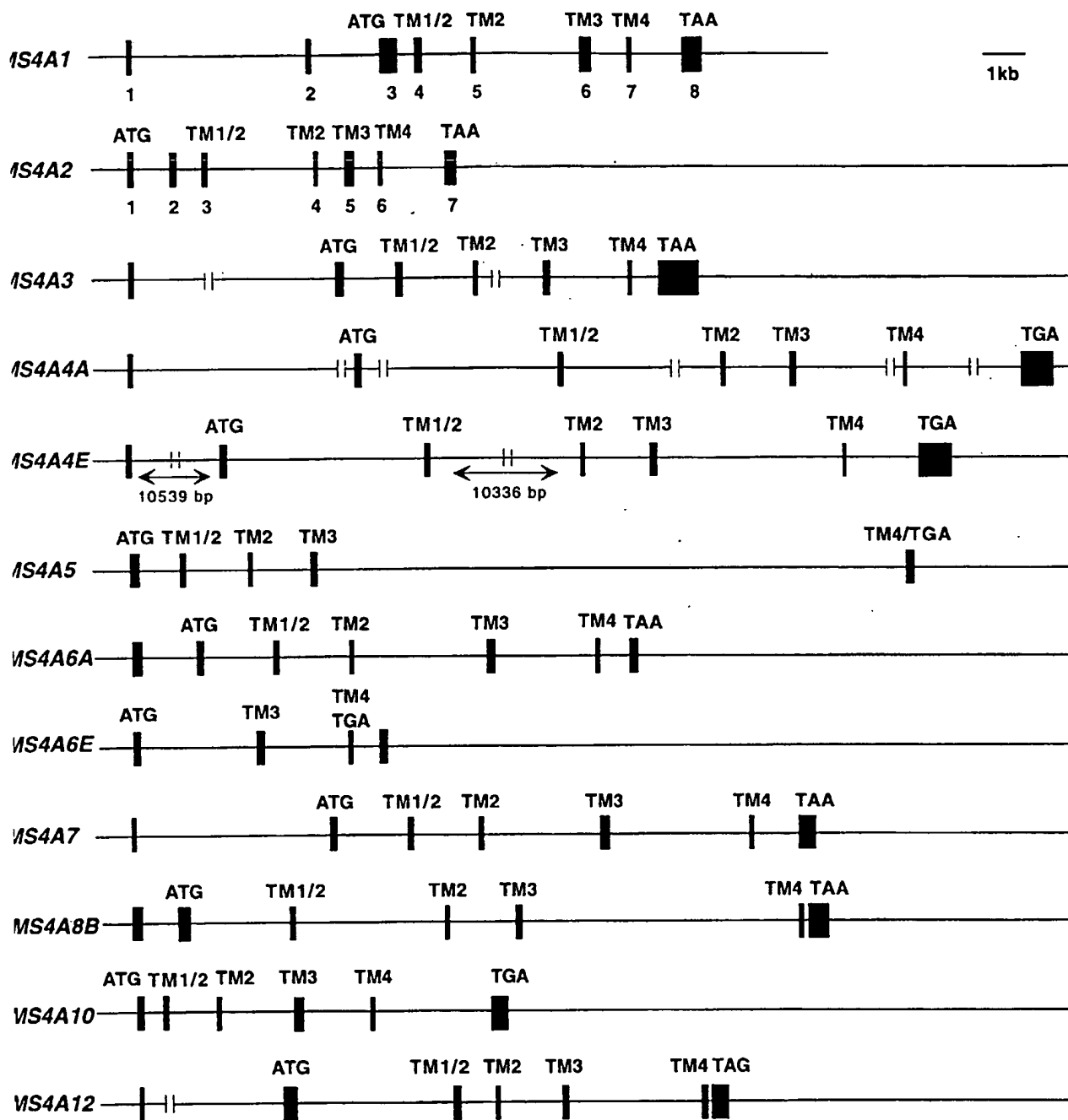


Figure 2

Figure 3

A4E ATCCGGGAAA TG GGGCT GTAGGAGAAT GGTGTCGAG AGAAGACAGA AAC TGTTAGT
 A4A ATGTGGGAAA TGGTGGGAAA GTGGGGGAAT AGTGTCTGAG AGAAGATAGA GACTATAAAA TGCTGTTCAT
 A4E GTGTGAGAAG ATTAGATGCT GTGGTGGGTT TGTGCTATT CTGTAAAGGT GGTTCATGGCC GGGGAGTGG AAC 3
 A4A GTGTGAGAAG ATTAGATGAT GTGGTGGGTT TGTGCTATT CTGTAAAGGT GGTTC CA AGAGGGGTGG AAC 3
 A4E ATT CCT GCA AAC GGG CTC AAC ATA TGC AAA TGT CTC AAT ATA GGA ATG AAA TTA CTT 60
 A4A ATT CCT GCA AAT GGT TTC AAT ATA TGC AGA TGT CTC GAT ATA GGA ATG AAA TTA CGT CTT 63
 A4E TAG AAC AAC TTA AAT AAG TCC AAT ATA CTT GGG GCT TTA AAA AAT AAA AAG GAG AAA TTC 120
 A4A TGG AAC AAC TTA AAT AAG TCA AAT ATA CTT GGA GCT TTA AA AAT TAA AAG GAG AGA GAT 122
 A4E AAG| ACC ACC TTT TCT GCT GCC ATG ACA ACC ATG CAA GGA ATG GAA CAG ACC ACT CCA GGG 180
 A4A TCG AG|C ACC TTT TCT GCT GCC ATG ACA ACC ATG CAA GGA ATG GAA CAG GCC ATG CCA GGG 182
 A4E P G P D V P Q L G N I D V I H S Y L C K
 A4E CCT GGC CCT GAT GTG CCC CAG CTG GGA AAC ATA CAT GTC ATA CAT TCA TAT CTG TGT AAA 240
 A4A CCT GGC CCT GGT GTG CCC CAG CTG GGA AAC ATG GCT GTC ATA CAT TCA CAT CTG TGG AAA 242
 A4E G L Q E K F F K R K P K V L G V V R I L
 A4E GGA TTG CAA GAG AAG TTC TTC AAG AGG AAA CCC AAA GTC CTT GGG|GTT GTG GGG ATT CTG 300
 A4A GGA TTG CAA GAG AAG TTC TTC AAG GGA GAA CCC AAA GTC CTT GGG|GTT GTG CAG ATT CTG 302
 A4E I A L H S L S H G I I H M C V A F S S Y
 A4E ATT GCC TTG ATG AGC CTT AGC ATG GGA ATA ATA ATG ATG TGT GTT GCA TTT AGT TCT TAT 360
 A4A ACT GCC CTG ATG AGC CTT AGC ATG GGA ATA ACA ATG ATG TGT ATG GCA TCT AAT ACT TAT 362
 A4E E E H P I F V Y V A Y T I W G S V M F I
 A4E GAA GAA CAT CCC ATT TTT GTG TAT GTT GCG TAC ACA ATT TGG GGG TCA GTG ATG|TTT ATT 420
 A4A GGA AGT AAC CCT ATT TCC GTG TAT ATC GGG TAC ACA ATT TGG GGG TCA GTA ATG|TTT ATT 422
 A4E I S V S L S V A A G I R T T K G L V G G
 A4E ATT TCA GTA TCC TTA TCA GTT GCA GCA GGA ATT AGA ACA ACA AAA GGT CTG|GTT GGA GGT 480
 A4A ATT TCA GGA TCC TTG TCA ATT GCA GCA GGA ATT AGA ACT ACA AAA GGC CTG|GTC CGA GGT 482
 A4E S L G K N I T S S V E A I S G I L I N A
 A4E AGT CTA GGA AAG AAT ATC ACC AGT TCA GTC TTG GCT ATA TCA GGG ATC TTA ATC AAT GCA 540
 A4A AGT CTA GGA ATG AAT ATC ACC AGC TCT GTA CTG GCT GCA TCA GGG ATC TTA ATC AAC ACA 542
 A4E I S L T F Y S F R Y H Y C N H D Q L S S
 A4E ATA AGC TTG AGC TTT TAT TCA TTC CGT TAC CAT TAC TGT AAC CAC GAT CAG TTG TCA AGT 600
 A4A TTT AGC TTG GCG TTT TAT TCA TTC CAT CAC CCT TAC TGT AAC TAC TAT GGC AAC TCA AAT 602
 A4E N C Y H T M S I L M G T D G M V L L L S
 A4E AAT TGT TAC ATG ACT ATG TCC ATT TTA ATG|GGT AGG GAT GGC ATG GTG CTC CTC TTA AGT 660
 A4A AAT TGT CAT GGG ACT ATG TCC ATC TTA ATG|GGT CTG GAT GGC ATG GTG CTC CTC TTA AGT 662
 A4E V L E F C I A V S L S A F G C K V L C C
 A4E GTG CTG GAA TTC TGC ATT GCT GTG TCC CTC TCT GCC TTT GGA TGT AAA GTG CTT TGT TGT 720
 A4A GTG CTG GAA TTC TGC ATT GCT GTG TCC CTC TCT GCC TTT GGA TGT AAA GTG CTC TGT TGT 722
 A4E S P S E F V L I T P S N S H M A E I A S
 A4E AGC CCC AGT GAG|TTT GTG CTA ATT ACA CCA TCA AAT TCT CAC ATG GCA GAA ATA GCA TCT 780
 A4A ACC CCT GGT GGG|GTT GTG TTA ATT CTG CCA TCA CAT TCT CAC ATG GCA GAA ACA GCA TCT 782
 A4E P T P L K T V
 A4E CCC ACA CCA CTT AAG ACA GTT TGA TGCCACCAAA AGATTAAACAG AAGAATGCTC CAGAAATCTA 844
 A4A CCC ACA CCA CTT AAT GAG GTT TGA TGCCACCAAA AGATTAAACAG ACAAATGCTC CAGAAATCTA 846
 A4E TGCTGACTGT AACACAAGAA CC CACATGA GAAAGTACCA GAATCCAACT CCAATACCTA TAGACATATT 913
 A4A TGCTGACTGT GACACAAGAG CCTCACAATGA GAAATTACCA GTATCCAACT TCGATACCTA TAGACTGTGT 916
 A4E GATATCATTA TTATATGGAA TCCAATTATG ACCTCTGTGT GTGTGTGTGT GTGTATATAT ATATATACAT 983
 A4A GATATTAATTA TTATATGTAA TCCAATTATG AACTGTGTGT GTATA 961
 A4E ATATATATAT ATACATATAT ATATATGTGT GTGTGTGTAT ATATTCAAAA TTTTGTCTC ATTTTTCCTC 1053
 A4A GAGAGAT|AAT AAA|TCAAAA TTATGTCTC ATTTTTCCTC 1001
 A4E CTGGAATCTA ACAACTAATT TCATTGGCCC TTTATCGAGA GTACTAGAAG TTAATTAAT|AAT|AAT|AATGCA 1123
 A4A CTGGAATCTA ATAACCTATT TCACTGGCTC TTTATCGAGA GTACTAGAAG TTAATTAAT|AAT|AAT|AATGCA 1071
 A4E TTTAATGAGG CAGCAGCACT TGAAGGTTT TCATTCACTA TTAGGACTTT ATATAAGGC ATTAACCTGGC 1194
 A4A TTTAATGAGG CAGCAGCACT TGAAGGTTT TCATTCACTA TAAGAACTTT ATATAAGGC ATTACATTGGC 1142
 A4E AAATAAGATT TGAAGCAGA AGGCCAAAA GGTATTG CTAAAACGAG GTCTCCATGC AAA CACATA 1259
 A4A AAATAAGGTT TGAAGCAGA AGGCCAAAA AAGATATTG TTAATAAGAG GCCTCCATGC AAACACATA 1212
 A4E CTCTCTCCCT CCTGTATAAC ATTCTCTCA CTTACTTGAC TTTTCTCTG CCATATTTGG GGACCAAGT 1329
 A4A CTCTCTCCCT C ATTTAT TTA ACTT TTTTCTCTC CTACCTATGG GGACCAAGT 1265
 A4E GCTTTTCTCT TCATGAAGTG GAGATTCAATG CCCCTCTCCC CCTCTCTTT CCTTCT GCTTCTCTTC 1395
 A4A GCTTTTCTCT TCAGGAAGTG GAGATTCAATG GCCATCTCCC CCTCTCTTT TCTTCTCTCT GCTTCTCTTC 1335
 A4E ACCCATAGAA AGTACCTTGG AATAGTATAG TCAGTCCCTG CATGTGCACA AGCTATCAT TCAGTAAAGG 1465
 A4A ACCCATAGAA AGTACCTTGA AGTAGCACAG TCCGTCTTGG CATGTGCACG AGCTATCAT TGAGTAAAGG 1405
 A4E TATACATGGA GTAAAAATCA TATGAAGGAT CAGATTCAAC TTATATTTC TATTTTCTC TCTTCTCTC 1535
 A4A TATACATGGA GTAAAAATCA TATTAAGCAT CAGATTCAAC TTATATTTC TATTTCTCTC TCTTCTCTC 1475
 A4E CCTTCCCTCC CCTTCTCTG GGCAGAAATTA TATCTTAATC AAATGTGTAT CCTGTGTAC ATATGGAAT 1605
 A4A CCTTCTCCCA CCTTCTCTG GGCATAATTA TATCTTAATC ATATATGGA AAATGTCAAC ATATGGAAT 1544
 A4E GTGCAACATA TGCTATTGT TAATGTTGT TAATACATT TGCTTTTTA TTGCAGAGCA AA|AATAAA|AT 1675
 A4A TGT TAAATAGC TTTCTTTTA TTGCAGAGCA AA|AATAAA|ATC 1585
 A4E TAGAAG CAATCTTTC ATGT 1695
 A4A AAATTAGAAG CAATAAA|AAA AAAAAAAAAA AAAA 1619

A6E							M	T	S	Q	P	I	S	N	E	T	I	I	M	
A6E		TT	GGC	AAC	ACC	ATT	ATG	ACA	TCA	CAA	CCT	ATT	TCC	AAT	GAG	ACC	ATC	ATA	ATG	53
A6A		TT	GGC	AAC	ACC	ATC	ATG	ACA	TCA	CAA	CCT	GTT	CCC	AAT	GAG	ACC	ATC	ATA	GTG	277
A6E	L	P	S	N	V	I	N	F	S	Q	A	E	K	P	E	P	T	N	Q	G
A6E	CTC	CCA	TCA	AAT	GTC	ATC	AAC	TTC	TCC	CAA	GCA	GAG	AAA	CCC	GAA	CCC	ACC	AAC	CAG	GGG
A6A	CTC	CCA	TCA	AAT	GTC	ATC	AAC	TTC	TCC	CAA	GCA	GAG	AAA	CCC	GAA	CCC	ACC	AAC	CAG	GGG
A6E	Q	D	S	L	K	X	R	L	Q	A	K	V	K	V	I	G				
A6E	CAG	GAT	AGC	CTG	CTG	AAG	AAA	CGT	CTA	CAG	GCA	AAA	GTC	AAA	<u>GTT ATT GGG</u>					161
A6A	CAG	GAT	AGC	CTG	AAG	AAA	CAT	CTA	CAC	GCA	GAA	ATT	AAA	<u>GTT ATT GGG</u>	(exons MS4A6A)					397
A6E	V	H	S	S	L	A	G	S	I	L	S	A	L	S	A	L	V	G	F	I
A6E	<u>GTG CAT AGC AGC CTG GCT GGA AGC ATT CTG AGT GCT CTG TCT GCC CTG GTG GGT TTC ATT</u>																			221
A6A	<u>GTG CAT AGC AGC CTG GTT GGA AGC ATT CTG AGT GCT CTG TCT GCC CTG GTG GGT TTC ATT</u>																			637
A6E	L	L	S	V	N	P	A	A	L	N	P	A	S	L	Q	C	K	L	D	E
A6E	<u>CTC CTG TCT GTC AAC CCG GCT GCA TTA</u>	AAT	CCT	GCC	TCA	TTG	CAG	TGT	AAG	TTG	GAC	GAA								281
A6A	<u>ATC CTG TCT GTC</u>	AAA	CAG	GCC	ACC	TTA	AAT	CCT	GCC	TCA	CTG	CAG	TGT	GAG	TTG	GAC	AAA			697
A6E	K	D	I	P	T	R	L	L	L	S	Y	D	Y	H		S	P	Y	T	M
A6E	AAG	GAT	ATA	CCA	ACC	AGA	CTT	CTT	CTT	TCT	TAT	GAT	TAT	CAT		TCA	CCT	TAC	ACC	ATG
A6A	AAT	AAT	ATA	CCA	ACA	AGA	AGT	TAT	GTT	TCT	TAT	CAT	TAT	CAT	GAT	TCA	CTT	TAC	ACC	ACG
A6E	D	C	H	R	A	K	A	S	L	A	G	T	L	S	L	M	L	V	S	T
A6E	GAC	TGC	CAT	AGA	GCC	AAA	GCC	AGT	<u>CTG GCT GGA ACT CTG TCT CTG ATG CTG GTT TCT ACT</u>											398
A6A	GAC	TGC	CAT	ACA	GCC	AAA	GCC	AGT	<u>CTG GCT GGA ACT CTC TCT CTG ATG CTG ATT TGC ACT</u>											817
A6E	V	L	E	F	C	L	A	V	L	T	A	V	L	Q	W	K	Q	T	V	*
A6E	<u>GTG TTG GAG TTC TGC CTA GCT GTG CTC ACT GCT GTG CTG</u>	CAG	TGG	AAA	CAG	ACT	GTC	TGA												458
A6A	<u>CTG CTG GAA TTC TGC CTA GCT GTG CTC ACT GCT GTG CTG</u>	CGG	TGG	AAA	CAG	GCT	TAC	TCT												877
A6E	C	TTT	CCT	GG													TTA	CTC	ATG	TCC
A6A	GAC	TTT	CCT	GGG	AGT	GTA	CTT	TTC	CTG	CCT	CAC	AGT	TAC	ATT	GGT	AAT	TCT	GGC	ATG	TCC
A6E	TCA	AAG	ACG	ACT	CAT	GAT	GCT	GGA	TAT	GAA	GAA	CTA	TTG	ACT	TCT	TGG	GAA	AAA	ACG	GAG
A6A	TCA	AAA	ATG	ACT	CAT	GAC	TGT	GGA	TAT	GAA	GAA	CTA	TTG	ACT	TCT	TAA	GAA	AAA	AGG	GAG
A6E	AAA	TAT	TAA	TTG	GAA	AGT	TGA	TTT	TTA	TGA	TAA	TAT	GGA	AAA	CCT	AAC	CAT	TAT	AAA	AAA
A6A	AAA	TAT	TAA	TCA	GAA	AGT	TGA	TTC	TTA	TGA	TAA	TAT	GGA	AAA	GTT	AAC	CAT	TAT	AGA	AAA
A6E	GCA	AA	CTT	GAG	TTT	CCT	AAA	TGT	AAG	CAT	TTA	AAG	TAA	ATG	CAT	ATT	TGT	TTT	AAA	AAA
A6A	GCA	AAG	CTT	GAG	TTT	CCT	AAA	TGT</												

A10	C CAG GGC CCC CAT CCA GCA TCA																			22		
a10	C CAG GGT TCC CGG CCA GGG TCA																			74		
A10	M	K	A	E		A	T	V	I	P	S	R	C	A	R	G	L	P	S	W		
A10	ATG	AAA	GCA	GAA		GCC	ACA	GTT	ATT	CCC	AGC	CGT	TGT	GCT	AGG	GGG	CTC	CCA	TCA	TGG	79	
a10	ATG	GCT	GGC	CAA	GCT	CCC	ACA	GCG	GTT	CCC	GGC	AGT	GTT	ACT	GGA	GAA	GTC	TCA	CCA	TGG	134	
A10	Q	V	L	S	P	V	Q	P	W	Q	T	S	A	P	Q	N	T	T	Q	P		
A10	CAA	GTC	CTC	AGC	CCA	GTC	CAG	CCC	TGG	CAG	ACA	AGT	GCA	CCC	CAG	AAC	ACG	ACC	CAG	CCC	139	
a10	CAG	AAC	CTA	GGG	CCT	GCC	CAG	CCT	GCA	CAG	AAA	GTA					GCC	CAG	CCC	179		
A10	K	L	L	A	P	H	Q	H		E	K	S	Q	K	K	S	S	L	L	K		
A10	AAG	CTC	CTG	GCT	CCA	CAC	CAG	CAC		GAG	AAG	TCC	CAG	AAG	AAG	AGC	AGC	CTT	CTT	AAG	196	
a10	CAA	AAC	CTG	GTT	CCA	GAT	GGG	CAC	CTT	GAG	AAA	GCC	CTG	GAG	GGA	AGT	GAC	CTT	CTA	CAG	239	
A10	E	L	G	A	F	H	I	T	I	A	L	L	H	L	V	F	G	G	Y	L		
A10	GAG	CTG	GGG	GCC	TTC	CAC	ATC	ACC	ATC	GCT	CTG	CTG	CAC	CTG	GTC	TTT	GGG	GGC	TAC	CTG	256	
a10	AAG	TTG	GGT	GGC	TTT	CAC	ATC	GCC	ATT	GCT	TTT	GCA	CAC	TTG	GCC	TTT	GGT	GGC	TAC	TTG	299	
A10	A	S	I	V	K	N	L	H	L	V	V	L	K	S	W	Y	P	F	W	G		
A10	GCC	TCT	ATA	GTC	AAG	AAC	CTT	CAC	CTG	GTG	GTG	CTG	AAG	TCT	TGG	TAT	CCA	TTC	TGG	GGG	316	
a10	ATC	TCC	ACA	GTC	AAG	AAT	CTG	CAC	CTG	GTG	GTG	TTG	AAG	TGC	TGG	TAT	CCA	CTC	TGG	GGA	359	
A10	A	A	S	F	L	I	S	G	I	L	A	I	T	M	K	T	F	S	K	T		
A10	GCT	GCC	TCT	TTT	CTC	ATT	TCA	GGG	ATC	TTG	GCG	GCG	ATA	ACA	ATG	AAG	ACC	TTT	TCT	AAA	ACT	376
a10	ACT	GCT	TCT	TTT	CTC	GTT	GCA	GGG	ATG	GCG	GCC	ATG	ACA	ACA	GTC	ACA	TTC	CCT	AAG	ACC	419	
A10	Y	L	K	M	L	C	L	M	T	N	L	I	S	L	F	C	V	L	S	G		
A10	TAC	CTG	AAG	ATG	TTG	TGC	CTG	ATG	ACA	AAC	CTC	ATC	AGC	CTC	TTT	TGC	GTG	CTG	TCT	GGC	436	
a10	TCT	CTG	AAG	GTT	CTC	TGT	GTG	ATA	GCC	AAC	GTT	ATC	AGC	TTG	TTC	TGC	GCA	CTG	GCC	GGC	479	
A10	L	F	V	I	S	K	D	L	F	L	E	S	P	F	E	S	P	I	W	R		
A10	CTC	TTC	GTC	ATC	TCC	AAG	GAT	CTC	TTT	CTG	GAG	AGC	CCA	TTT	GAG	TCC	CCG	ATC	TGG	AGA	496	
a10	TTT	TTT	GTC	ATT	GCC	AAG	GAC	CTC	TTC	CTG	GAG	GGT	CCT	TTT	CCA	TGG	CCA	ATC	TGG	AGA	539	
A10	M	Y	P	N	S	T	V	H	I	Q	R	L	E	L	A	L	L	C	F	T		
A10	ATG	TAC	CCC	AAC	TCC	ACG	GTC	CAC	ATC	CAG	AGG	CTG	GAG	CTG	GCC	TTG	CTC	TGC	TTC	ACT	556	
a10	CCA	TAC	CCT	GAA	CCC	ACA	ACC	TAC	ATC	CAA	AGG	CTA	GAG	CTG	ACC	CTG	TTC	TGC	TTC	ACC	599	
A10	V	L	E	L	F	L	P	V	P	T	A	V	T	A	W	R	G	D	C	P		
A10	GTC	CTA	GAG	CTC	TTC	CTG	CCA	GTC	CCC	ACA	GCT	GTC	ACA	GCC	TGG	AGA	GGG	GAG	TGC	CCA	616	
a10	TTT	CTG	GAG	ATC	TTT	CTG	TCG	GGG	TCC	ACA	GCC	ATC	ACA	GCC	TAT	AGG	ATG	AAA	CGC	CTG	659	
A10	S	A	K		N	D	D	A	C	L	V	P	N	T	P	L	H	L	K	G		
A10	TCT	GCA	AAG		AAT	GAT	GAT	GCA	TGC	CTT	GTT	CCG	AAT	ACA	CCA	TTG	CAT	CTC	AAA	GGC	673	
a10	CAA	GCA	GAG	GAC	AAG	GAT	GAC	ACC	CCT	TTC	GTT	CCT	GAC	ACA	CCT	ATG	GAA	CTT	AAA	GGT	719	
A10	L	P	V	E	P	P	P	S	Y	Q	S	V	I	Q	G	D	A	Q	H	K		
A10	CTG	CCG	GTG	GAG	CCC	CCG	CCA	TCC	TAC	CAG	AGT	GTG	ATT	CAA	GGC	GAC	GCA	CAA	CAC	AAG	733	
a10	CTA	TCG	CTG	GGG	CCA	CCA	CCA	TCC	TAT	AAA	GAT	GTG	GCC	CAA	GGA	CAC	TCC	TCC	TCT	GAT	779	
A10	Q	H	Q	R	*																	
A10	CAA	CAT	CAG	AGG	TGA	AGA	GGT	TTG	GCC	CTG	GCT	CCC	CAG	ACC	TTC	AGA	ACC	CAC	GCT	TGG	793	
a10	ACT	GGC	AGA	GCC	TTG	GCC	ACC	AGC	TCT	GGA	CTG	CTG	CTG	GCC	TCT	GAC	TCC	TTC	CAT	CAA	839	
A10	CCC	CTT	CTC	CCT	GGC	ATA	GAT	AGA	AAC	CAG	TTC	CCA	GGG	GAGCTGTAAG	GGGCAAGGGG						852	
a10	GCT	CTG	CTT	CAC	ACG	GGC	CCA	AGG	ACC	TTG	AGA	AAG	TAG	TCTAAGACTA	CTAGACTTTTC						898	
A10	CTAACAGTGC	TGCAGCTAGA	ACTGCCTGGC	CTGGGAGTGG	TAGCCAAGGC	AGCAATGGTC	CTATCCCCCTC														922	
a10	CCAAATAA	GA	CTGAGTTCTT	GCAATGCCAA	GTCCCTTCCCT	GCCTCCCATT	GGCTGGACTC	CGGTGATGAC													968	
A10	AGGCACTAGA	GGAACCTCCCA	GGGCCCCCCA	GGAACATCTC	AGCACCTGAC																972	
a10	ACAGCGCTGA	CTTGACAA	AA	TAAA	TGCATA	ACCACTATGA	AAAAAAAAA														101	

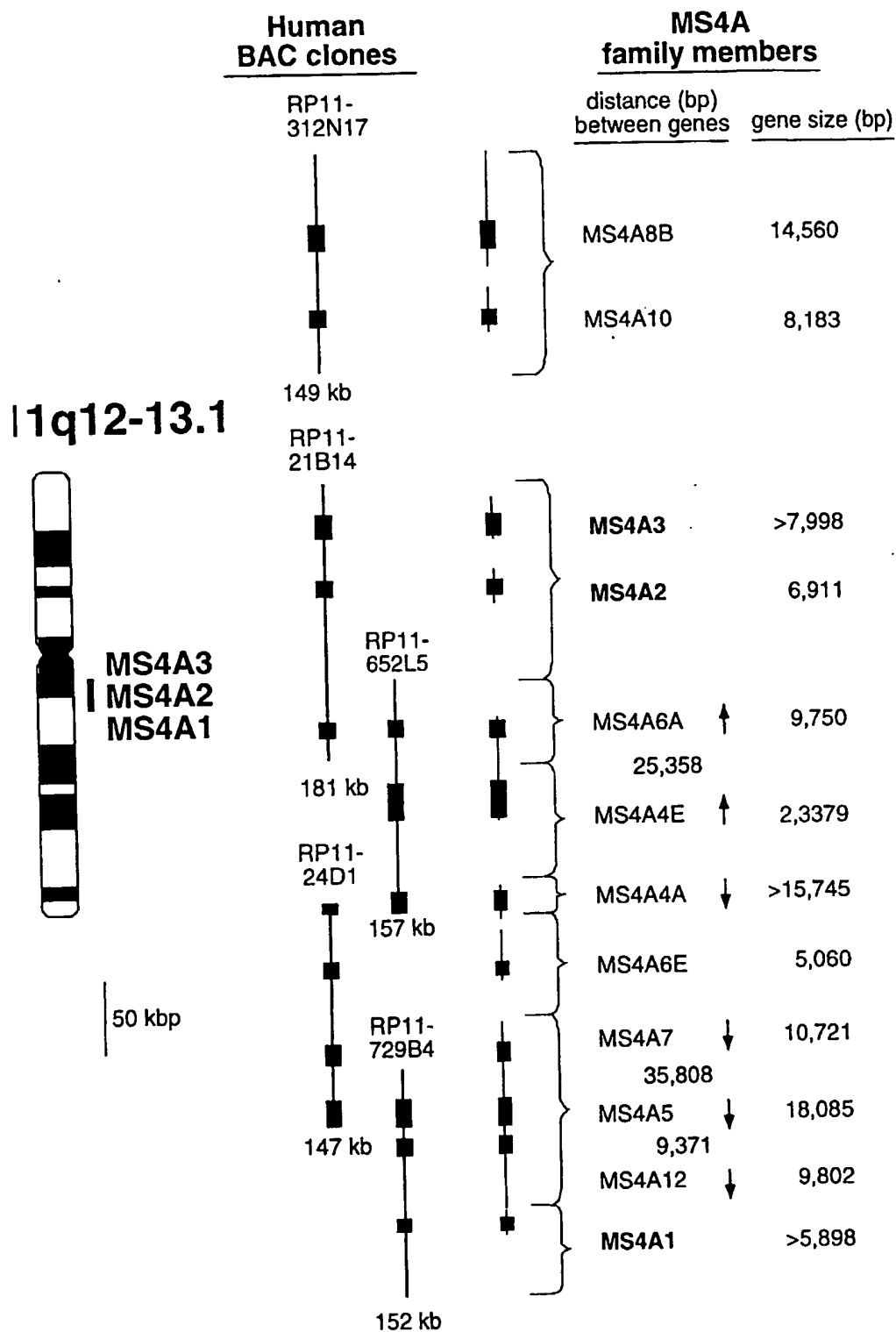
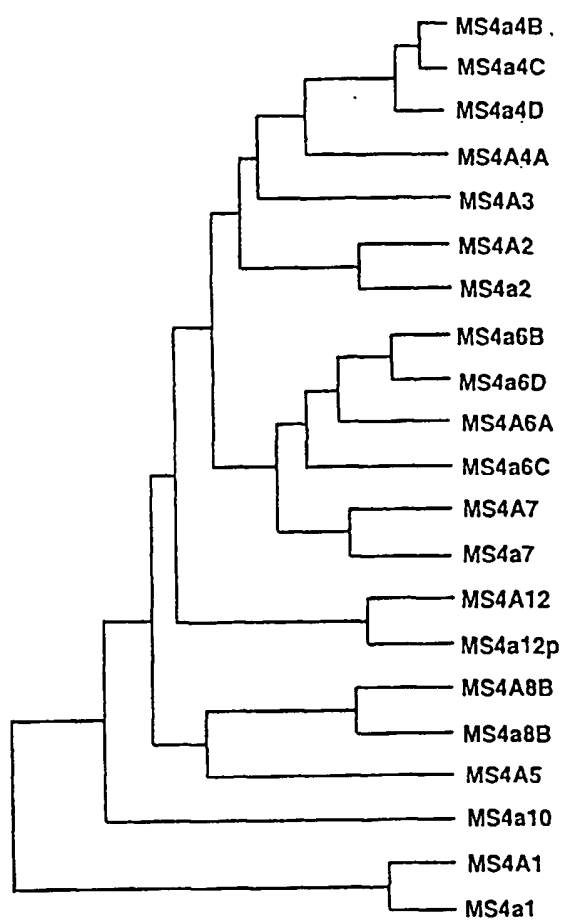


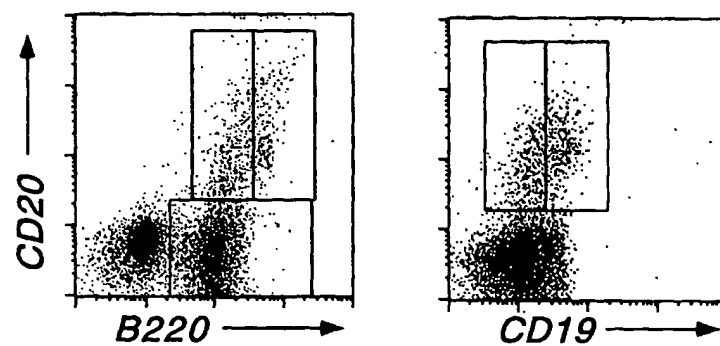
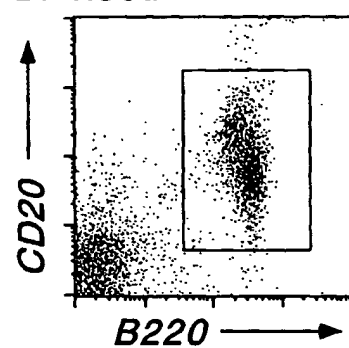
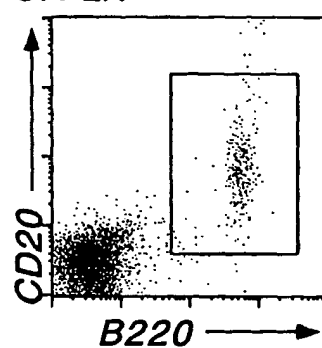
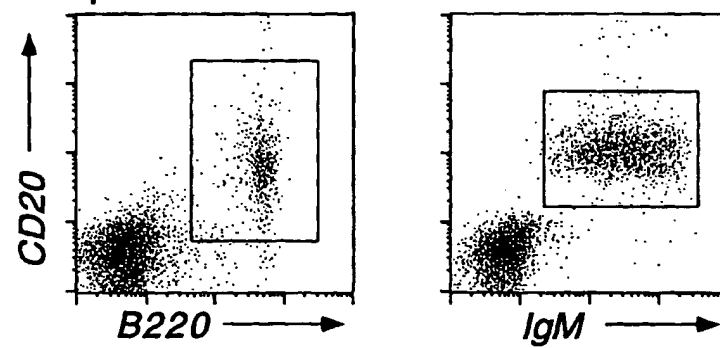
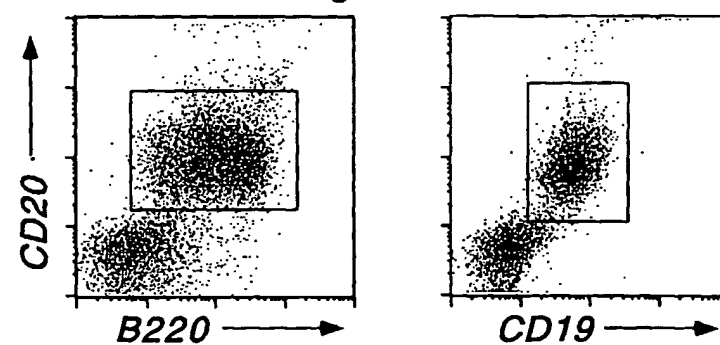
Figure 6

Figure 7

		-----TM1-----			
A1	MTTPRNSVNGTTPAEPNKGPIAMQSGPKPLFRHSSLSVGPTQSPFMRESKTLG	AVQIMNGLFHIILAGGLMIPAGI	76		
A1	MSGPPAEPTKGLFAMQGPAPKVNLRKTSLSLVGPTQSPFMRESKALG	AVQIMNGLFHIITLGLGLMIPGV	69		
A2	MDTESNRRANLALP QEPSSIVPAPEVLEISPOEVSSGRLLKSSAPPLHTWLTVMKQDEFLG	VTQILTAHILCLCPGTIVCSVLV	85		
A2	MDTENRSRADLALPQESS SAPDILLEASP	AKAAPPKQWRTFPLKKELEFLG	77		
A3	MASHEVDNABLGASAHGTPGSETGPEELMTSVYHPINGSPDYQKAKLVGL	AIQILNAAMIILALGIFLVCLOV	75		
A3		MKPEETGGSVYQPLDESRHVQRGVQLAG	52		
A4A	MTTMOQMOPAGPGVQPLGNMVAIHSHLAKGLQEKPLKGEKVLG	VVQILTAHLSLMSGIMTMOMASN	71		
A4B	MOCQDQTTMAVPGVAVPSKNSVMTSQMNKEKKEPLKGEKVLG	VLQVMIAIINLSGLTIIITLTF	67		
A4C	MOCQDQTTMAVPGGAPPSESVKSMQMNENKEKPLKGEKVLG	VVQVMIAIINLSGLTIIINLNS	67		
A4D	MOCQDQTTMAVPGGAPPSESVKSMQMNENKEKPLKGEKVLG	AIQVMIAIINLSGLTIIINLNV	67		
A4E	MTTMOQMBOPTTGGPGDPVQPLQNDIVHISYLCGLQEKPLKGEKVLG	VVRILIAHLSLMSGIMTMCVAFS	71		
A5	MDSSTAHSPVFLVFPFEITASEYESTELSATFTSTQSPGLKPLFARKMKIIG	TIQILFGIMTSPFGVIFLPTLLK	74		
A6A	MTSQPVVNETIIVLPSNVNFSQAEKPEPTNQGDLSLQGLHABIKVIG	TIQILCGMMVLSGLIILASAFS	72		
A6B	MIPOVVTSETVAMISFNGMSLPQTDKQPFQWQDLSLQGLHABIKVMA	AIQIMCAVMVLSGLIILASVPSN	72		
A6C	MIPOVVTNETITTFISFNGINFPQKDESQPTQQRDLSLQGLHABIKVIV	AIQIMCAVTLSGLIILASVPSN	72		
A6D	MIPOVVTSETVTVISFNGISFPQTDKQPFQWQDLSLQGLHABIKVMA	AIQIMCAVMVLSGLIILASVPSN	72		
A6E	MTSQPISNETIIMLPSNVNFSQAEKPEPTNQGDLSLQGLHABIKVIV				
A7	MLQSDQTMGVSHSPTKGIITIPQREKPGHMYQNEEDYLQNLQPTETTTLG	TVQILCLLIISSGLAILVFPAPY	72		
A7	MRLLQGTQIHGMDCFPKDIIMHREKTGHTYKEDDLLIGVPSEATLIG	TIQILCALILASFGGILVSASY	71		
A8B	MNSMTSAVVPANSVVLVAVPHNGYPTVPTGIMSHVPLVINSQPVHLPVGNPPSLVSNVQGPVQKALKEGKTLG	AIQIIILGLAHILGSGIMATVLVG	96		
A8B	MEPEQERLTQPGTVMNTVSPGPMANSVYVAVPNSYVVPVGTVPQMP	YPSNQPVHVISGLHPLGVPMTEPPAQRLVKGGQVLG	112		
A10	MKAEA TVIPSCRARGLPMSQVLSVPQWQTSAPQNTQPKLLAPHOH	EKSQKSSSLKELG	84		
A10	MAQQAAPTAVPGSVTGEVSRKQNLGPAQPAQKVA	QPONLVDPGHLEKALEGSDLLQKLG	81		
A12	MMSSKPTSHAEVNETIPNPYPGFSFMAPGQPLGSLNLENQAQGAQRAQPIGTSPGIPASSQPGQGNIQHINPSVGTAVMNFKEEAKALG	VIIQIMVGLMHIGGIVLCLISPS	115		
A12	MMSSKPTTYPGVYGTTPDLYPPSNFMVPGSQQPGFINPRIQVQSSQ	APFIVSGPINNSQVQGNIQHINVPQGTGAATNFKEEAKTLG	112		
		-----TM2-----			
A1	YAPICVTV	WYPLWGGIM	YIISGSLAA TEONSRKCL	182	
A1	FAPICLSV	WYPLWGGIM	YIISGSLAAAEKTSRKSL	176	
A2	SHIEGDISSPKA	GYPFWGAIF	FSGISGLMIIISERRNAT YL	VRGSLGANTASSIAGCTGITIIINLQKSL	171
A2	SDFOEVLVLLYL	GYPFWGAVL	FVLSGFLSIIISERON TLYL	VRGSLGAMNIVSSIAAGTGAMLIINLTNPF	162
A3	YHFOKHFPPFTFYT	GYPFWGAVF	PCSSGTLVAVAGIRP TRTW	IQNSPGMINASATIALVGTAFSLNIAVNI	162
A3	SHHFRHFPPFTFYT	GYPFWGAVF	FISGSLTVAVAGIRP TRML	MQNSPGMINASTTIAFVGTVFLSVHLAFN*	162
A4A	TYGSNPISVYI	GYTIWGSVM	FIIISGSLIAAGIRT TKGL	VRGSLGMINITSVLAASGILINTPSLAFYS	154
A4B	SELPISVVL	MVPIWGSIM	FIVSGSLIAAGVTP TKCL	IVASLTANTITSVLAATASINGVSVAVGS	137
A4C	SEPLISVVL	MAPIWGSIM	FIVSGSLIAAGVTP TKSL	IISSLTANTITSVLAATASINGVSVAVGS	137
A4D	SERFMSVLL	LAPFWGSIM	FIVSGSLIAAGVTP TKAM	IISSLSVNTISSVLAASIIIGVSVISGV	136
A4E	SYEHPFIVVY	AYTIWGSVM	FIIISVLSVAAGIRT TKGL	VGSLGMINITSVLAASGILINLSLTFYS	154
A5	YPPRFPFIFLS	GYPFWGSVL	FINSGLFIAVAGIRT TETL	IIISLRMINFSLALGAIAGIILITFGFIL	155
A6A	PNFTQVSTLLNS	AYPPIGPPF	FIIISGSLIAATEKRL TKLL	VHSLVGSIIISALSALVGPIILSVKQATLNPASLQ	174
A6B	LHFTSVFVLLKS	GYPPIGALF	FIVSGSLIAATEKRS TKIL	VDSSLTANILSVSFAFMGIIISVLSLGLHAPASDQ	170
A6C	PFYNSVPSVLLKS	GYPPIGALF	FIIISGSLIAATEKRS TKPL	VDSSLTANILSVSFAFMGIIISVLSLGLHAPASDQ	173
A6D	LHFTSVFVLLKS	GYPPIGALF	FIIISGSLIAATEKRS TKPL	VHSLVGSIIISALSALVGPIILSVLSLAALPALAQ	173
A6E				VHSLVGSIIISALSALVGPIILSVNPAALNPASLQ	109
A7	SHFNPASTTLM	GYPPIGALC	FCITGSLISIIISG KQSTKPP	DLSLTSNAVSSVTAAGALFLADSHVALR	173
A7	FNPEVSTTLIS	GYPPIGSLC	FAIAGILSIIISXIS TKPF	ALSSLASNAVSSVAVIGLFLFTYCLIALG	170
A8B	EYLSISFYC	GYPFWGGLW	FIIISGSLVAAEQNPYSYCL	LSGSLGLNIVSAICSAVGVILFTDLSIPH	174
A8B	HYTPVSLYC	GYPFWGGLW	FIIISGSLVAAEQNPYSYCL	LNGSVGLNIVSAICSAVGVILFTDLSIPH	174
A10	LHLVVLKS	WYPMKGAAS	FLISGILAIITKRTFS KTYL	IKMLCLMTNLIISLFCVLSGLFVISKDLFLES	192
A10	LHLVVLKS	WYPMKGAAS	FLISGILAIITKRTFS KTYL	IKMLCLMTNLIISLFCVLSGLFVISKDLFLES	164
A10	LHLVVLKS	WYPMKGAAS	FLISGILAIITKRTFS KTYL	IKMLCLMTNLIISLFCVLSGLFVISKDLFLES	161
A12	PREVLGPASTAVIG	GYPFWGGLS	FIIISGSLVSAKSEL SRCL	VKSLGMINIVSSIIAFIIGVILLVDMCING	192
A12	YMQVLGPASLAFVS	GYPFWGGLS	FIITGILCILASKS SPAL	IKSLGMINIVSSIIAFIIGVILLVDMCING	189
		-----TM3-----			
A1	CYSIQSLFL	GILSVMLIPAFPOELVIAGIVENEMKRTCSRKKS	NIIVLLSAEKKQETIIEIKEEVWGLTETSQPKNEEDIIEIIPQEEEEEETETNFPPEPQDQESSPIENDSSP297		
A1	CNSIQSVPL	GILSVMLIPAFPOELVIAGIVENEMKRTCSRKKS	NIIVLLSAEKKQETIIEIKEEVWGLTETSQPKNEEDIIEIIPQEEEEEETETNFPPEPQDQESSPIENDSSP297		
A2	CFMASPST	EIVVMMLFLTILGLGSAVSLTICGAGEELKGNK	VPEDRVYEELNIIYSATYSELEDPGEMSPPIDL	244	
A2	C FVASPTT	ELVVMMLFLTILAFCSAVLFTIYRIQOELESKK	VPEDRVYEELNIIYSATYSELEDPGEMSPPIDL	235	
A3	CNYMGSISN	QMSVLLILTLLELCVTITSTIAMNCCNCCNSRE	EISSPPNSV	214	
A4A	CHGTMSILM	GLDQVLLLSVLEFCIAVLSAFCCKVLCCPTGG	VVLILPSHSHMAETASPTPLNEV	220	
A4B	FRYNYITIK	GLDVIMLIPMLLEFCIAVLSAFCCKVCCNSRE	VVLVLPSPNPVETMAPMTLQPLPSEHQGTNVPQNVYKHPGEIV	226	
A4C	FRYNYITIK	GLDVIMLIPMLLEFCIAVLSAFCCKVCCNSRE	VVLVLPSPNPVETMAPMTLQPLPSEHQGTNVPQNVYKHPGEIV	226	
A4D	QFRSQAIA	SLDVIMLIPMLLEFCIAVLSAFCCKVCCNSRE	VVLVLPSPNPVETMAPMTLQPLPSEHQGTNVPQNVYKHPGEIV	226	
A4E	CYMTMSILM	GLDQVLLLSVLEFCIAVLSAFCCKVCCNSRE	FVLITPSNISHMAETASPTPLKTV	220	
A5	CKAVTVLPL	GILITLMTFPIELFISLPPSILGCHSEDCDCDCDC		200	
A6A	CYTAASLA	GTLISLMLICTLLEFCIAVLTAVLRNKQAYSDPPG	SVLFLPHSYIINQSMSSIMTHDCGYEELTS	248	
A6B	CFAAKSVLA	GVPISLMLICTLLEFCIAVLTAVLRNKQSHSNIPG	NVMFLPHSSNDSNMSKVLNCPSYEEQLVC	244	
A6C	CAVTKSILT	GALSVMILISVLELGLALLSAHLWREGVLTSLRM		217	
A6D	CPVAKAALT	GVPISLMLISVLELGLALLSAHLWREGVLTSLRM	NVIFLSONSKKSSVSSSELNCPYENILTS	247	
A6E	CHRAKASLA	GTLISLMLISVLELGLALLSAHLWREGVLTSLRM		247	
A7	CLITSVSLT	GLVVMMLIPVLELGLALLSAHLWREGVLTSLRM	SSFSSTQSDHIQVQKSSSRSHI	230	
A7	CYLAVVMAM	SALGQMLLPTVLEFLAGYSSIFMKQVYSNKP	GTFFLPQSDQHTQLVSNLILQ	244	
A8B	ANGV	IRPGMAISGVLLVFCLEFGIACASSHFCQCLVCCQSSN	VSIVYP NIYAANPVITPEPVSPPSYSEIQANK	250	
A8B	ENLGV	RTGVAISSVLLVFCLELISASVSHFCQVACCHYNN	PGVVIPI NVYAANPVITPEPVSPPSYSEIQANK	260	
A10		VHIORELELALCLPTVLEFLPVPVAVTAMRGDCPSAK	NDACLVPTPLKLCGLPVEPPSYQSVICQDAHQHQR	241	
A10		TYIORELELALCLPTVLEFLPVPVAVTAMRGDCPSAK	NDACLVPTPLKLCGLPVEPPSYQSVICQDAHQHQR	267	
A12	DYNAV	LSGKGISATLMIISLLEFFVACATAHFANQANTTMM	SVLVI PMYESNPVTPASSAPPCNNYSANAPK	267	
A12	DYNAV	LSGKGISATLMIISLLEFFVACATAHFANQANTTMM	SVLVI PMYESNPVTPASSAPPCNNYSANAPK	243	

Figure 8



A. Bone Marrow**B. Blood****C. PLN****D. Spleen****E. Peritoneal Lavage****Figure 9**

10/12

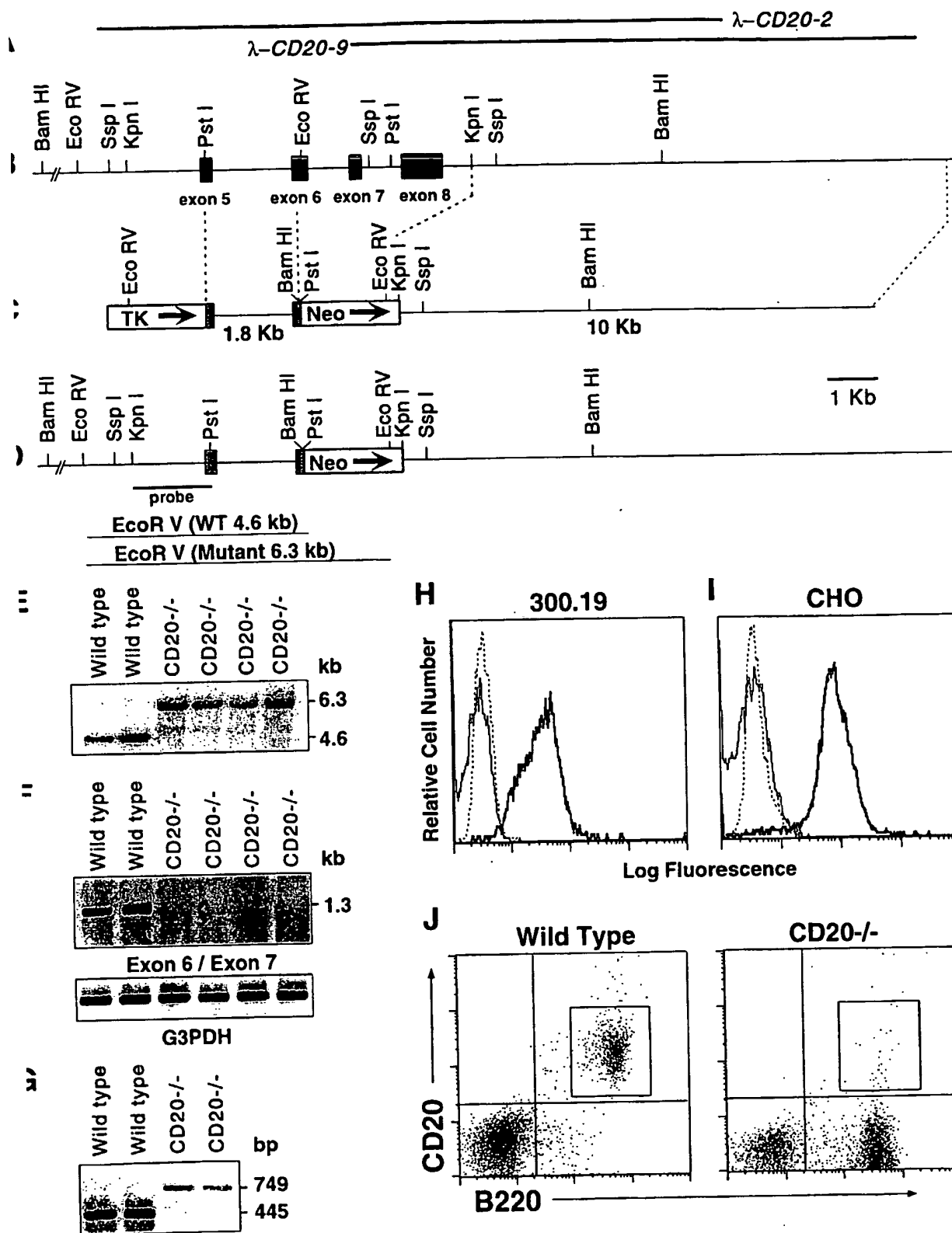


Figure 10

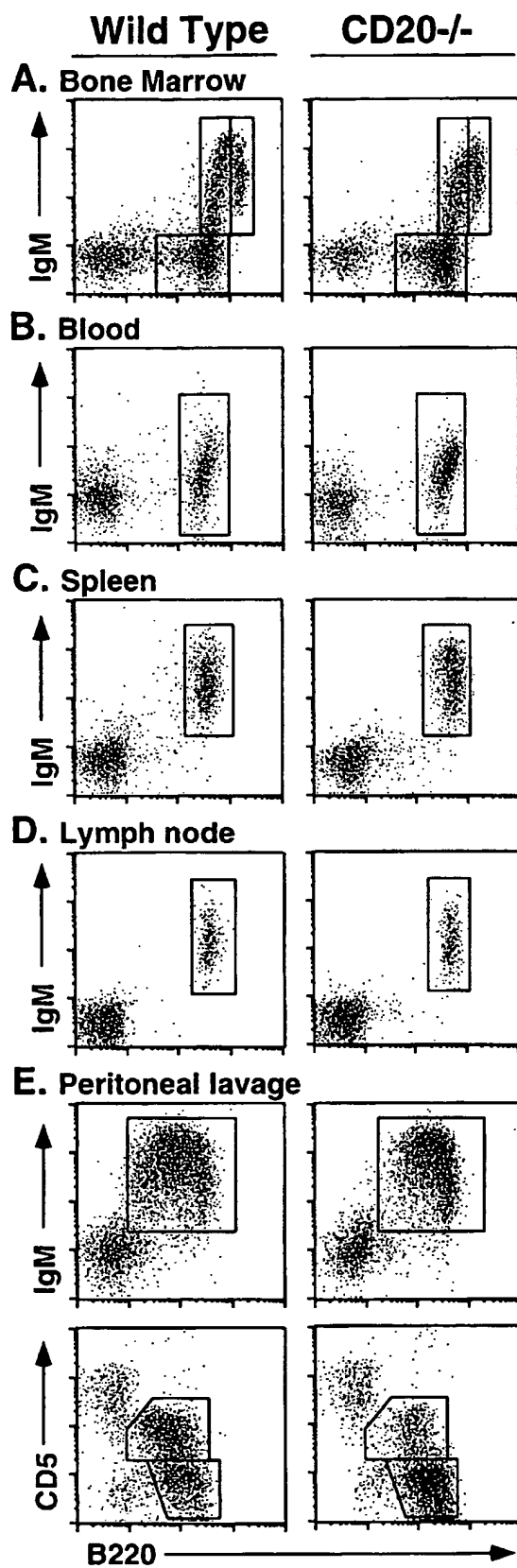


Figure 11

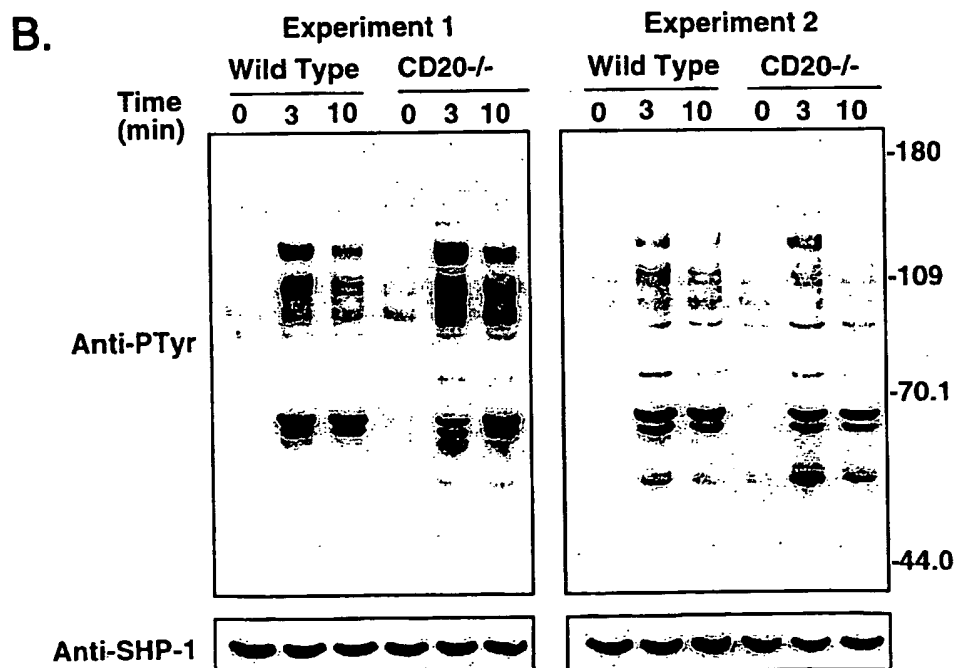
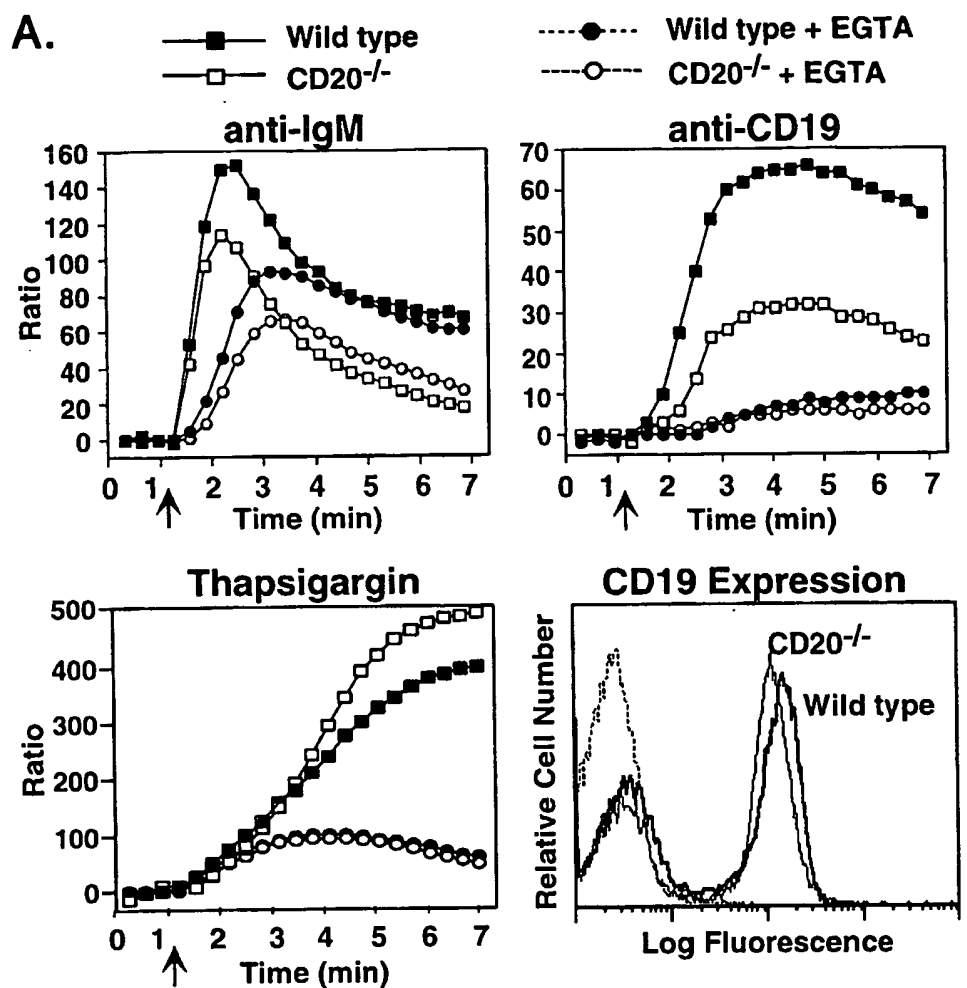


Figure 12

Sequence Listing

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	Glu Pro Lys Val Leu Gly Val Val Gln Ile Leu Thr Ala Leu Met Ser	
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	Ser Asn Pro Ile Ser Val Tyr Ile Gly Tyr Thr Ile Trp Gly Ser Val	
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30	gta ctg gct gca tca ggg atc tta atc aac aca ttt agc ttg gcg ttt	557
	Val Leu Ala Ala Ser Gly Ile Leu Ile Asn Thr Phe Ser Leu Ala Phe	
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Leu Gly Thr Ile Gln Ile Leu Phe Gly Ile Met Thr Phe Ser Phe Gly
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115 120 125

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Ala Gly Ile Ile Leu Leu Thr Phe Gly Phe Ile Leu Asp Gln Asn Tyr
130 135 140 145

att tgt ggt tat tct cac caa aat agt cag tgt aag gct gtt act gtc 537
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	35 40 45	
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	130 135 140	
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	245	
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Tyr Val Ser Tyr Phe Tyr His Asp Ser Leu Tyr Thr Thr Asp Cys Tyr
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35	cca aca aga agt tat gtt tct tac ttt tat cat gat tca ctt tat acc Pro Thr Arg Ser Tyr Val Ser Tyr Phe Tyr His Asp Ser Leu Tyr Thr	160	165	170	532
40	acg gac tgc tat aca gcc aaa gcc agt ctg gct gga tct ctc tct ctg Thr Asp Cys Tyr Thr Ala Lys Ala Ser Leu Ala Gly Ser Leu Ser Leu	175	180	185	580
45	atg ctg att tgc act ctg ctg gaa ttc tgc cta gct gtg ctc act gct Met Leu Ile Cys Thr Leu Leu Glu Phe Cys Leu Ala Val Leu Thr Ala	190	195	200	628
50	gtg ctg cgg tgg aaa cag gct tac tct gac ttc cct ggg agt gta ctt Val Leu Arg Trp Lys Gln Ala Tyr Ser Asp Phe Pro Gly Ser Val Leu	205	210	215	676
55	ttc ctg cct cac agt tac att ggt aat tct ggc atg tcc tca aaa atg Phe Leu Pro His Ser Tyr Ile Gly Asn Ser Gly Met Ser Ser Lys Met	225	230	235	724
60	act cat gac tgt gga tat gaa gaa cta ttg act tct taa gaaaaaagg Thr His Asp Cys Gly Tyr Glu Glu Leu Leu Thr Ser	240	245		773
	agaaatatta atcagaaagt tgattcttat gataatatgg aaaagttaac cattatagaa				833
	aagcaaagct tgagtttctt aaatgtaagc ttttaaagta atgaacatta aaaaaaacca				893
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 35 40 45
 25 Gly Thr Ile Gln Ile Leu Cys Gly Met Met Val Leu Ser Leu Gly Ile
 50 55 60
 30 Ile Leu Ala Ser Ala Ser Phe Ser Pro Asn Phe Thr Gln Val Thr Ser
 65 70 75 80
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 85 90 95
 40 Ile Ser Gly Ser Leu Ser Ile Ala Thr Glu Lys Arg Leu Thr Lys Leu
 100 105 110
 45 Leu Val His Ser Ser Leu Val Gly Ser Ile Leu Ser Ala Leu Ser Ala
 115 120 125
 50 Leu Val Gly Phe Ile Ile Leu Ser Val Lys Gln Ala Thr Leu Asn Pro
 130 135 140
 55 Ala Ser Leu Gln Cys Glu Leu Asp Lys Asn Asn Ile Pro Thr Arg Ser
 145 150 155 160
 60 Tyr Val Ser Tyr Phe Tyr His Asp Ser Leu Tyr Thr Thr Asp Cys Tyr
 165 170 175
 65 Thr Ala Lys Ala Ser Leu Ala Gly Ser Leu Ser Leu Met Leu Ile Cys
 180 185 190
 70 Thr Leu Leu Glu Phe Cys Leu Ala Val Leu Thr Ala Val Leu Arg Trp
 195 200 205

Lys Gln Ala Tyr Ser Asp Phe Pro Gly Ser Val Leu Phe Leu Pro His
 210 215 220

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Ser Tyr Ile Gly Asn Ser Gly Met Ser Ser Lys Met Thr His Asp Cys
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Gly Tyr Glu Glu Leu Leu Thr Ser
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atg ctc cca tca aat gtc atc aac ttc tcc caa gca gag aaa ccc gaa 98
 Met Leu Pro Ser Asn Val Ile Asn Phe Ser Gln Ala Glu Lys Pro Glu
 15 20 25

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ccc acc aac cag ggg cag gat agc ctg aag aaa cgt cta cag gca aaa 146
 Pro Thr Asn Gln Gly Gln Asp Ser Leu Lys Lys Arg Leu Gln Ala Lys
 30 35 40

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gtc aaa gtt att ggg gtg cat agc agc ctg gct gga agc att ctg agt 194
 Val Lys Val Ile Gly Val His Ser Ser Leu Ala Gly Ser Ile Leu Ser
 45 50 55 60

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gct ctg tct gcc ctg gtg ggt ttc att ctc ctg tct gtc aac ccg gct 242
 Ala Leu Ser Ala Leu Val Gly Phe Ile Leu Leu Ser Val Asn Pro Ala
 65 70 75

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gca tta aat cct gcc tca ttg cag tgt aag ttg gac gaa aag gat ata 290
 Ala Leu Asn Pro Ala Ser Leu Gln Cys Lys Leu Asp Glu Lys Asp Ile
 80 85 90

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cca acc aga ctt ctt ctt tct tat gat tat cat tca cct tac acc atg 338
 Pro Thr Arg Leu Leu Leu Ser Tyr Asp Tyr His Ser Pro Tyr Thr Met
 95 100 105

gac tgc cat aga gcc aaa gcc agt ctg gct gga act ctg tct ctg atg 386
 Asp Cys His Arg Ala Lys Ala Ser Leu Ala Gly Thr Leu Ser Leu Met
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ctg gtt tct act gtg ttg gag ttc tgc cta gct gtg ctc act gct gtg 434
 Leu Val Ser Thr Val Leu Glu Phe Cys Leu Ala Val Leu Thr Ala Val
 125 130 135 140

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ctg cag tgg aaa cag act gtc tga cttccctggt tactcatgtc ctcaaagacg 488
 Leu Gln Trp Lys Gln Thr Val
 145

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actcatgatg ctggatatga agaactattg acttcttggg aaaaaacgga gaaatattaa 548
 ttggaaagtt gatttttatg ataatatgga aaacctaacc attataaaaa agcaaacttg 608
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Asn Val Ile Asn Phe Ser Gln Ala Glu Lys Pro Glu Pro Thr Asn Gln
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40

Gly Gln Asp Ser Leu Lys Lys Arg Leu Gln Ala Lys Val Lys Val Ile
 35 40 45

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Gly Val His Ser Ser Leu Ala Gly Ser Ile Leu Ser Ala Leu Ser Ala
 50 55 60

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Leu Val Gly Phe Ile Leu Leu Ser Val Asn Pro Ala Ala Leu Asn Pro
 65 70 75 80

Ala Ser Leu Gln Cys Lys Leu Asp Glu Lys Asp Ile Pro Thr Arg Leu
 85 90 95

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Leu Leu Ser Tyr Asp Tyr His Ser Pro Tyr Thr Met Asp Cys His Arg
 100 105 110

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Ala Lys Ala Ser Leu Ala Gly Thr Leu Ser Leu Met Leu Val Ser Thr

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	atg cta tta caa tcc caa acc atg ggg gtt tct cac agc ttt aca cca	165		
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	Lys Gly Ile Thr Ile Pro Gln Arg Glu Lys Pro Gly His Met Tyr Gln			
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	Asn Glu Asp Tyr Leu Gln Asn Gly Leu Pro Thr Glu Thr Thr Val Leu			
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	Gly Thr Val Gln Ile Leu Cys Cys Leu Leu Ile Ser Ser Leu Gly Ala			
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	Ile Leu Val Phe Ala Pro Tyr Pro Ser His Phe Asn Pro Ala Ile Ser			
	65 70 75 80			

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15	ttt gac ctg agc agc ttg acc tca aat gca gtg agt tct gtt act gca Phe Asp Leu Ser Ser Leu Thr Ser Asn Ala Val Ser Ser Val Thr Ala 115 120 125	501
20	gga gca ggc ctc ttc ctc ctt gct gac agc atg gta gcc ctg agg act Gly Ala Gly Leu Phe Leu Leu Ala Asp Ser Met Val Ala Leu Arg Thr 130 135 140	549
25	gcc tct caa cat tgt ggc tca gaa atg gat tat cta tcc tca ttg cct Ala Ser Gln His Cys Gly Ser Glu Met Asp Tyr Leu Ser Ser Leu Pro 145 150 155 160	597
30	tat tcg gag tac tat tat cca ata tat gaa atc aaa gat tgt ctc ctg Tyr Ser Glu Tyr Tyr Tyr Pro Ile Tyr Glu Ile Lys Asp Cys Leu Leu 165 170 175	645
35	acc agt gtc agt tta aca ggt gtc cta gtg gtg atg ctc atc ttc act Thr Ser Val Ser Leu Thr Gly Val Leu Val Val Met Leu Ile Phe Thr 180 185 190	693
40	gtg ctg gag ctc tta tta gct gca tac agt tct gtc ttt tgg tgg aaa Val Leu Glu Leu Leu Leu Ala Ala Tyr Ser Ser Val Phe Trp Trp Lys 195 200 205	741
45	cag ctc tac tcc aac aac cct ggg agt tca ttt tcc tcg acc cag tca Gln Leu Tyr Ser Asn Asn Pro Gly Ser Ser Phe Ser Ser Thr Gln Ser 210 215 220	789
50	caa gat cat atc caa cag gtc aaa aag agt tct tca cgg tct tgg ata Gln Asp His Ile Gln Gln Val Lys Lys Ser Ser Ser Arg Ser Trp Ile 225 230 235 240	837
55	taa gtaactcttg gcctcagagg aaggaaaagc aactcaacac tcatgggtcaa gtgtgattag actttcctga aatctctgcc attttagata ctgtgaaaca aactaaaaaa aaaaaagctt ttgttttgta tttgtttact atgagtcgtt atttaatttc tcttgaaaat aatttcctca aagcccaagt caataaatgt tatcagccag tcttccaaaa tggtcataaa ctttataaac tgctttgggt aaactgagca gaaggtgata cacagaaggg aaaatgtgca ctcatgctag tgtgaatttg gtaagtcgag tgactctgca ggctgtttct gtattatttt cacactcata ttgcttaaatt attacatatt agggattg	890 950 1010 1070 1130 1190 1228
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 35 40 45
 25 Gly Thr Val Gln Ile Leu Cys Cys Leu Leu Ile Ser Ser Leu Gly Ala
 50 55 60
 Ile Leu Val Phe Ala Pro Tyr Pro Ser His Phe Asn Pro Ala Ile Ser
 65 70 75 80
 30 Thr Thr Leu Met Ser Gly Tyr Pro Phe Leu Gly Ala Leu Cys Phe Gly
 85 90 95
 35 Ile Thr Gly Ser Leu Ser Ile Ile Ser Gly Lys Gln Ser Thr Lys Pro
 100 105 110
 40 Phe Asp Leu Ser Ser Leu Thr Ser Asn Ala Val Ser Ser Val Thr Ala
 115 120 125
 45 Gly Ala Gly Leu Phe Leu Leu Ala Asp Ser Met Val Ala Leu Arg Thr
 130 135 140
 Ala Ser Gln His Cys Gly Ser Glu Met Asp Tyr Leu Ser Ser Leu Pro
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 50 Tyr Ser Glu Tyr Tyr Tyr Pro Ile Tyr Glu Ile Lys Asp Cys Leu Leu
 165 170 175
 55 Thr Ser Val Ser Leu Thr Gly Val Leu Val Val Met Leu Ile Phe Thr
 180 185 190
 60 Val Leu Glu Leu Leu Leu Ala Ala Tyr Ser Ser Val Phe Trp Trp Lys
 195 200 205

Gln Leu Tyr Ser Asn Asn Pro Gly Ser Ser Phe Ser Ser Thr Gln Ser
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 acgtggctgc tggcagagca agc atg aat tcg atg act tca gca gtt ccg gtg 233
 Met Asn Ser Met Thr Ser Ala Val Pro Val
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gcc aat tct gtg ttg gtg gtg gca ccc cac aat ggt tat cct gtg acc 281
 Ala Asn Ser Val Leu Val Val Ala Pro His Asn Gly Tyr Pro Val Thr
 15 20 25

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cca gga att atg tct cac gtg ccc ctg tat cca aac agc cag ccg caa 329
 Pro Gly Ile Met Ser His Val Pro Leu Tyr Pro Asn Ser Gln Pro Gln
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gtc cac cta gtt cct ggg aac cca cct agt ttg gtg tcg aat gtg aat 377
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 45 50 55

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ggg cag cct gtg cag aaa gct ctg aaa gaa ggc aaa acc ttg ggg gcc 425
 Gly Gln Pro Val Gln Lys Ala Leu Lys Glu Gly Lys Thr Leu Gly Ala
 60 65 70

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	Ile Gln Ile Ile Ile Gly Leu Ala His Ile Gly Leu Gly Ser Ile Met	
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10	gcg acg gtt ctc gta ggg gaa tac ctg tct att tca ttc tac gga ggc	521
	Ala Thr Val Leu Val Gly Glu Tyr Leu Ser Ile Ser Phe Tyr Gly Gly	
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15	ttt ccc ttc tgg gga ggc ttg tgg ttt atc att tca gga tct ctc tcc	569
	Phe Pro Phe Trp Gly Gly Leu Trp Phe Ile Ile Ser Gly Ser Leu Ser	
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	Val Ala Ala Glu Asn Gln Pro Tyr Ser Tyr Cys Leu Leu Ser Gly Ser	
	125 130 135	
25	ttg ggc ttg aac atc gtc agt gca atc tgc tct gca gtt gga gtc ata	665
	Leu Gly Leu Asn Ile Val Ser Ala Ile Cys Ser Ala Val Gly Val Ile	
	140 145 150	
30	ctc ttc atc aca gat cta agt att ccc cac cca tat gcc tac ccc gac	713
	Leu Phe Ile Thr Asp Leu Ser Ile Pro His Pro Tyr Ala Tyr Pro Asp	
	155 160 165 170	
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	Tyr Tyr Pro Tyr Trp Gly Val Asn Pro Gly Met Ala Ile Ser Gly	
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40	gtg ctg ctg gtc ttc tgc ctc ctg gag ttt ggc atc gca tgc gca tct	809
	Val Leu Leu Val Phe Cys Leu Leu Glu Phe Gly Ile Ala Cys Ala Ser	
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45	tcc cac ttt ggc tgc cag ttg gtc tgc tgt caa tca agc aat gtg agt	857
	Ser His Phe Gly Cys Gln Leu Val Cys Cys Gln Ser Ser Asn Val Ser	
	205 210 215	
50	gtc atc tat cca aac atc tat gca gca aac cca gtg atc acc cca gaa	905
	Val Ile Tyr Pro Asn Ile Tyr Ala Ala Asn Pro Val Ile Thr Pro Glu	
	220 225 230	
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	Pro Val Thr Ser Pro Pro Ser Tyr Ser Ser Glu Ile Gln Ala Asn Lys	
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	ccctttctgg gcttccataa cccaggtcgt tctgttctg acagctgagg aaacgtctct	1066
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 35 40 45
 30 Asn Pro Pro Ser Leu Val Ser Asn Val Asn Gly Gln Pro Val Gln Lys
 50 55 60
 35 Ala Leu Lys Glu Gly Lys Thr Leu Gly Ala Ile Gln Ile Ile Ile Gly
 65 70 75 80
 40 Leu Ala His Ile Gly Leu Gly Ser Ile Met Ala Thr Val Leu Val Gly
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 45 Glu Tyr Leu Ser Ile Ser Phe Tyr Gly Gly Phe Pro Phe Trp Gly Gly
 100 105 110
 50 Leu Trp Phe Ile Ile Ser Gly Ser Leu Ser Val Ala Ala Glu Asn Gln
 115 120 125
 55 Pro Tyr Ser Tyr Cys Leu Leu Ser Gly Ser Leu Gly Leu Asn Ile Val
 130 135 140
 60 Ser Ala Ile Cys Ser Ala Val Gly Val Ile Leu Phe Ile Thr Asp Leu
 145 150 155 160
 55 Ser Ile Pro His Pro Tyr Ala Tyr Pro Asp Tyr Tyr Pro Tyr Ala Trp
 165 170 175
 60 Gly Val Asn Pro Gly Met Ala Ile Ser Gly Val Leu Leu Val Phe Cys
 180 185 190

5 Leu Leu Glu Phe Gly Ile Ala Cys Ala Ser Ser His Phe Gly Cys Gln
 195 200 205
 10 Leu Val Cys Cys Gln Ser Ser Asn Val Ser Val Ile Tyr Pro Asn Ile
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 Pro Ser Trp Gln Val Leu Ser Pro Val Gln Pro Trp Gln Thr Ser Ala
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 Pro Gln Asn Thr Thr Gln Pro Lys Leu Leu Ala Pro His Gln His Glu
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 Lys Ser Gln Lys Lys Ser Ser Leu Leu Lys Glu Leu Gly Ala Phe His
 50 55 60
 55 atc acc atc gct ctg ctg cac ctg gtc ttt ggg ggc tac ctg gcc tct 240
 Ile Thr Ile Ala Leu Leu His Leu Val Phe Gly Gly Tyr Leu Ala Ser
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 60 ata gtc aag aac ctt cac ctg gtg gtg ctg aag tct tgg tat cca ttc 288
 Ile Val Lys Asn Leu His Leu Val Val Leu Lys Ser Trp Tyr Pro Phe
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	115 120 125	
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	Leu Ile Ser Leu Phe Cys Val Leu Ser Gly Leu Phe Val Ile Ser Lys	
	130 135 140	
15	gat ctc ttt ctg gag agc cca ttt gag tcc ccg atc tgg aga atg tac	480
	Asp Leu Phe Leu Glu Ser Pro Phe Glu Ser Pro Ile Trp Arg Met Tyr	
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	165 170 175	
25	ttc act gtc cta gag ctc ttc ctg cca gtg ccc aca gct gtc aca gcc	576
	Phe Thr Val Leu Glu Leu Phe Leu Pro Val Pro Thr Ala Val Thr Ala	
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	Trp Arg Gly Asp Cys Pro Ser Ala Lys Asn Asp Asp Ala Cys Leu Val	
	195 200 205	
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	Pro Asn Thr Pro Leu His Leu Lys Gly Leu Pro Val Glu Pro Pro Pro	
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	Arg	
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70	Pro Gln Asn Thr Thr Gln Pro Lys Leu Leu Ala Pro His Gln His Glu	

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25	Lys Thr Phe Ser Lys Thr Tyr Leu Lys Met Leu Cys Leu Met Thr Asn 115 120 125		
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25 caaggtggta cctcatcagg cagacac atg aag cca gag gag act ggt ggt tct 174

Met Lys Pro Glu Glu Thr Gly Gly Ser

1 5

30 gtt tat cag ccc ttg gat gag tca cgc cat gtt caa cga ggt gta ctg 222

Val Tyr Gln Pro Leu Asp Glu Ser Arg His Val Gln Arg Gly Val Leu

10 15 20 25

35 caa gcc ctc ggg gcc atc cag atc ctg aat gga ata ctg att cta gct 270

Gln Ala Leu Gly Ala Ile Gln Ile Leu Asn Gly Ile Leu Ile Leu Ala

30 35 40

40 ctc gga att ttt ctg gtt tgt tta caa cac gtg tcc cac cac ttc agg 318

Leu Gly Ile Phe Leu Val Cys Leu Gln His Val Ser His His Phe Arg

45 50 55

cat ttc ttc ttc ttc acc ttc tac aca ggc tac cca ctg tgg ggt gct 366

His Phe Phe Phe Phe Thr Phe Tyr Thr Gly Tyr Pro Leu Trp Gly Ala

60 65 70

45 gtg ttt ttt atc agc tca gga tcc ttg act gtt gcc gca ggg aga aac 414

Val Phe Phe Ile Ser Ser Gly Ser Leu Thr Val Ala Ala Gly Arg Asn

75 80 85

50 ccc aca cga atg ctg atg caa aac agt ttt ggg ata aac att gcc agt 462

Pro Thr Arg Met Leu Met Gln Asn Ser Phe Gly Ile Asn Ile Ala Ser

90 95 100 105

55 act aca att gca ttt gtt ggg act gtt ttc ctt tct gtg cat ttg gca 510

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110 115 120

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Phe Asn

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 35 40 45
 Leu Gln His Val Ser His His Phe Arg His Phe Phe Phe Phe Thr Phe
 50 55 60
 30 Tyr Thr Gly Tyr Pro Leu Trp Gly Ala Val Phe Phe Ile Ser Ser Gly
 65 70 75 80
 35 Ser Leu Thr Val Ala Ala Gly Arg Asn Pro Thr Arg Met Leu Met Gln
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 40 Asn Ser Phe Gly Ile Asn Ile Ala Ser Thr Thr Ile Ala Phe Val Gly
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Met Gln Gly Gln Glu Gln
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20 acc acc atg gca gtg gtt cct gga gtt gct gtg cct tca aag aat tct 163
Thr Thr Met Ala Val Val Pro Gly Val Ala Val Pro Ser Lys Asn Ser
10 15 20

25 gtt atg aca tca caa atg tgg aat gag aag aaa gag aaa ttc ttg aag 211
Val Met Thr Ser Gln Met Trp Asn Glu Lys Lys Glu Lys Phe Leu Lys
25 30 35

30 ggg gaa ccc aaa gtc ctt ggg gtt tta caa gtg atg att gct atc ata 259
Gly Glu Pro Lys Val Leu Gly Val Leu Gln Val Met Ile Ala Ile Ile
40 45 50

35 aac ctc agc tta gga ata ata att ttg aca act tta ttt tct gaa cta 307
Asn Leu Ser Leu Gly Ile Ile Ile Leu Thr Thr Leu Phe Ser Glu Leu
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ccc act tca gtg atg tta atg gtc cca att tgg gga tca ata atg ttc 355
Pro Thr Ser Val Met Leu Met Val Pro Ile Trp Gly Ser Ile Met Phe
75 80 85

40 att gtc tcc gga tcc ctg tcc att gca gca gga gtg aca cct aca aaa 403
Ile Val Ser Gly Ser Leu Ser Ile Ala Ala Gly Val Thr Pro Thr Lys
90 95 100

45 tgc ctg atc gtt gcc agt cta act ctg aac act atc acc tct gtg ttg 451
Cys Leu Ile Val Ala Ser Leu Thr Leu Asn Thr Ile Thr Ser Val Leu
105 110 115

50 gct gca act gca agc ata atg ggt gta gtc agt gtg gct gtg ggt tca 499
Ala Ala Thr Ala Ser Ile Met Gly Val Val Ser Val Ala Val Gly Ser
120 125 130

55 cag ttt ccg ttt cgg tat aat tat aca atc acc aag ggt ttg gat gtt 547
Gln Phe Pro Phe Arg Tyr Asn Tyr Thr Ile Thr Lys Gly Leu Asp Val
135 140 145 150

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Leu Met Leu Ile Phe Asn Met Leu Glu Phe Cys Leu Ala Val Ser Val
155 160 165

60 tct gca ttt gga tgt gaa gct tcc tgt tgt aac tcc cgt gag gtt ctt 643
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15	gga aat gtg tac aag aac cac cca gga gaa ata gtc taa ttttgatgtg Gly Asn Val Tyr Lys Asn His Pro Gly Glu Ile Val 215 220 225	788		
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25	tagatgcttt tgatcaagta caaactcata aagtatgtaa gaaattacta atgataggaa ccaattttta tctcatatgt aacatagtggt ataatttaat agatctggta aaaatttata ataaagagaa atgcctgaaa	1148 1208 1228		
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60	Val Met Ile Ala Ile Ile Asn Leu Ser Leu Gly Ile Ile Ile Leu Thr 50 55 60			
65	Thr Leu Phe Ser Glu Leu Pro Thr Ser Val Met Leu Met Val Pro Ile 65 70 75 80			

5 Trp Gly Ser Ile Met Phe Ile Val Ser Gly Ser Leu Ser Ile Ala Ala
 85 90 95
 Gly Val Thr Pro Thr Lys Cys Leu Ile Val Ala Ser Leu Thr Leu Asn
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 10 Thr Ile Thr Ser Val Leu Ala Ala Thr Ala Ser Ile Met Gly Val Val
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 15 Ser Val Ala Val Gly Ser Gln Phe Pro Phe Arg Tyr Asn Tyr Thr Ile
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 20 Thr Lys Gly Leu Asp Val Leu Met Leu Ile Phe Asn Met Leu Glu Phe
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 Cys Leu Ala Val Ser Val Ser Ala Phe Gly Cys Glu Ala Ser Cys Cys
 165 170 175
 25 Asn Ser Arg Glu Val Leu Val Val Leu Pro Ser Asn Pro Val Glu Thr
 180 185 190
 30 Val Met Ala Pro Pro Met Thr Leu Gln Pro Leu Leu Pro Ser Glu His
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Met Gln Gly
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15 cag gaa cag acc acc atg gca gtg gtt cct gga ggt gct cca cct tca 163
Gln Glu Gln Thr Thr Met Ala Val Val Pro Gly Gly Ala Pro Pro Ser
5 10 15

20 gag aat tct gtt atg aaa tca caa atg tgg aac gag aac aag gag aaa 211
Glu Asn Ser Val Met Lys Ser Gln Met Trp Asn Glu Asn Lys Glu Lys
20 25 30 35

25 ttc ttg aag ggg gaa ccc aaa gtc ctt ggg gtt gta caa gtt atg att 259
Phe Leu Lys Gly Glu Pro Lys Val Leu Gly Val Val Gln Val Met Ile
40 45 50

30 gct ctc ata aac ctc agc ttc gga ata ata att ttg gca aat cta tct 307
Ala Leu Ile Asn Leu Ser Phe Gly Ile Ile Ile Leu Ala Asn Leu Ser
55 60 65

35 tct gaa cca ctc att tct gtg gtc tta atg gct cca att tgg gga cca 355
Ser Glu Pro Leu Ile Ser Val Val Leu Met Ala Pro Ile Trp Gly Pro
70 75 80

40 ata atg ttc att gtc tca gga tcc ctg tca att gca gca gga gtg aga 403
Ile Met Phe Ile Val Ser Gly Ser Leu Ser Ile Ala Ala Gly Val Arg
85 90 95

cct aca aaa aag ctg atc atc agc agt cta act ctg aac act atc acc 451
Pro Thr Lys Lys Leu Ile Ile Ser Ser Leu Thr Leu Asn Thr Ile Thr
100 105 110 115

45 tct gtg ttg gct gca act gca agc ata atg ggt gta gtc agt gtg gct 499
Ser Val Leu Ala Ala Thr Ala Ser Ile Met Gly Val Val Ser Val Ala
120 125 130

50 gtg ggt tca cag ttt ccg ttt cgg tat aat tat aca atc acc aag ggt 547
Val Gly Ser Gln Phe Pro Phe Arg Tyr Asn Tyr Thr Ile Thr Lys Gly
135 140 145

55 ttg gat att ttg atg tta att tta aat atg cta gaa ttc tgc att gct 595
Leu Asp Ile Leu Met Leu Ile Leu Asn Met Leu Glu Phe Cys Ile Ala
150 155 160

60 gtg tcc atc tct gct ttt gga tgt aaa gct tcc tgt tgt aac tcc agc 643
Val Ser Ile Ser Ala Phe Gly Cys Lys Ala Ser Cys Cys Asn Ser Ser
165 170 175

gag gtt ctt gta gtg cta cca tca aat cct gct gtg act gtg atg gca 691

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10	aat	gtt	cca	gaa	aat	gta	tat	aag	aac	cac	tca	gaa	gaa	ata	gtc	taa	787		
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				20					25					30					
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60	Val	Met	Ile	Ala	Leu	Ile	Asn	Leu	Ser	Phe	Gly	Ile	Ile	Ile	Leu	Ala			

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10	Trp Gly Pro Ile Met Phe Ile Val Ser Gly Ser Leu Ser Ile Ala Ala 85 90 95		
15	Gly Val Arg Pro Thr Lys Lys Leu Ile Ile Ser Ser Leu Thr Leu Asn 100 105 110		
20	Thr Ile Thr Ser Val Leu Ala Ala Thr Ala Ser Ile Met Gly Val Val 115 120 125		
25	Ser Val Ala Val Gly Ser Gln Phe Pro Phe Arg Tyr Asn Tyr Thr Ile 130 135 140		
30	Thr Lys Gly Leu Asp Ile Leu Met Leu Ile Leu Asn Met Leu Glu Phe 145 150 155 160		
35	Cys Ile Ala Val Ser Ile Ser Ala Phe Gly Cys Lys Ala Ser Cys Cys 165 170 175		
40	Asn Ser Ser Glu Val Leu Val Val Leu Pro Ser Asn Pro Ala Val Thr 180 185 190		
45	Val Met Ala Pro Pro Val Thr Leu Gln Pro Leu Pro Pro Ser Glu His 195 200 205		
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Thr Thr Met Ala Val Val Pro Gly Gly Ala Pro Pro Ser Glu Asn Ser
10 15 20

25 gtt ata aaa tca caa atg tgg aac aag aac aaa gag aaa ttc ttg aag 149
Val Ile Lys Ser Gln Met Trp Asn Lys Asn Lys Glu Lys Phe Leu Lys
25 30 35

30 ggg gaa ccc aaa gtc ctc ggg gct ata caa gtt atg att gct ttc ata 197
Gly Glu Pro Lys Val Leu Gly Ala Ile Gln Val Met Ile Ala Phe Ile
40 45 50

35 aac ttc agc tta gga ata ata att ata tta aat aga gtt tct gaa cga 245
Asn Phe Ser Leu Gly Ile Ile Ile Ile Leu Asn Arg Val Ser Glu Arg
55 60 65 70

40 ttc atg tca gtg ctc tta ctg gcc cca ttt tgg gga tca ata atg ttc 293
Phe Met Ser Val Leu Leu Ala Pro Phe Trp Gly Ser Ile Met Phe
75 80 85

45 att ttc tca gga tcc ctg tca att gca gca gga gtg aaa cct aca aaa 341
Ile Phe Ser Gly Ser Leu Ser Ile Ala Ala Gly Val Lys Pro Thr Lys
90 95 100

50 gcc atg atc atc agc agt cta agt gtg aac act atc agt tct gtg ttg 389
Ala Met Ile Ile Ser Ser Leu Ser Val Asn Thr Ile Ser Ser Val Leu
105 110 115

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Ala Val Ala Ala Ser Ile Ile Gly Val Ile Ser Val Ile Ser Gly Val
120 125 130

60 ttt cgc caa ttt aga agt caa cca gcc atc gct agt ttg gat gtt ttg 485
Phe Arg Gln Phe Arg Ser Gln Pro Ala Ile Ala Ser Leu Asp Val Leu
135 140 145 150

65 atg aca att ttg aat atg cta gaa ttc tgc att gct gtg tcc gtc tct 533
Met Thr Ile Leu Asn Met Leu Glu Phe Cys Ile Ala Val Ser Val Ser
155 160 165

70 gca ttt ggg tgt aaa gct tcc tgt tgt aac tcc agt gag gtt ctt gta 581
Ala Phe Gly Cys Lys Ala Ser Cys Cys Asn Ser Ser Glu Val Leu Val
170 175 180

5 gtg cta cca tca aat tct gct gtg aca gtg aca gca ccc ccc atg ata 629
 Val Leu Pro Ser Asn Ser Ala Val Thr Val Thr Ala Pro Pro Met Ile
 185 190 195

10 ctt caa cca ttg cca cca tca gaa tgc caa ggg aaa aat gtt cca gaa 677
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 200 205 210

15 aat cta tac agg aac caa cca gga gaa ata gtc taa ttttgatgta 723
 Asn Leu Tyr Arg Asn Gln Pro Gly Glu Ile Val
 215 220 225

20 cgtgttttca tgtgtgagag tgtgtgtgtg cacatgtgta tgcatttggc tttttgtacg 783
 aatataagac atgctctaaa taagtaagtc aagcatttat taagtcaata acactttaaa 843
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55 Lys Glu Lys Phe Leu Lys Gly Glu Pro Lys Val Leu Gly Ala Ile Gln
 35 40 45

60 Val Met Ile Ala Phe Ile Asn Phe Ser Leu Gly Ile Ile Ile Ile Leu
 50 55 60

65 Asn Arg Val Ser Glu Arg Phe Met Ser Val Leu Leu Leu Ala Pro Phe
 65 70 75 80

5 Trp Gly Ser Ile Met Phe Ile Phe Ser Gly Ser Leu Ser Ile Ala Ala
 85 90 95

Gly Val Lys Pro Thr Lys Ala Met Ile Ile Ser Ser Leu Ser Val Asn
 100 105 110

10 Thr Ile Ser Ser Val Leu Ala Val Ala Ala Ser Ile Ile Gly Val Ile
 115 120 125

15 Ser Val Ile Ser Gly Val Phe Arg Gln Phe Arg Ser Gln Pro Ala Ile
 130 135 140

20 Ala Ser Leu Asp Val Leu Met Thr Ile Leu Asn Met Leu Glu Phe Cys
 145 150 155 160

25 Ile Ala Val Ser Val Ser Ala Phe Gly Cys Lys Ala Ser Cys Cys Asn
 165 170 175

Ser Ser Glu Val Leu Val Val Leu Pro Ser Asn Ser Ala Val Thr Val
 180 185 190

30 Thr Ala Pro Pro Met Ile Leu Gln Pro Leu Pro Pro Ser Glu Cys Gln
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15 gggagctcaa tc atg att cca caa gta gtg acc agt gag act gtg gca atg 171

Met Ile Pro Gln Val Val Thr Ser Glu Thr Val Ala Met

1 5 10

20 att tcg cca aat gga atg agt ctt ccc caa aca gac aaa ccc cag cct 219

Ile Ser Pro Asn Gly Met Ser Leu Pro Gln Thr Asp Lys Pro Gln Pro

15 20 25

25 ttc cac cag tgg caa gac agc ctg aag aaa cat cta aag gct gag atc 267

Phe His Gln Trp Gln Asp Ser Leu Lys Lys His Leu Lys Ala Glu Ile

30 35 40 45

30 aaa gtg atg gcg gca atc cag atc atg tgt gct gtg atg gtg ttg agt 315

Lys Val Met Ala Ala Ile Gln Ile Met Cys Ala Val Met Val Leu Ser

50 55 60

35 ctg gga atc att ttg gca tct gtt ccc tcc aat cta cac ttt acc tca 363

Leu Gly Ile Ile Leu Ala Ser Val Pro Ser Asn Leu His Phe Thr Ser

65 70 75

40 gtg ttt tca gtc ctg tta aaa tct ggc tac cca ttt ata gga gct ttg 411

Val Phe Ser Val Leu Leu Lys Ser Gly Tyr Pro Phe Ile Gly Ala Leu

80 85 90

45 ttt ttt ata gtc tct gga att ctg tcc atc gtc acg gag aca aag tca 459

Phe Phe Ile Val Ser Gly Ile Leu Ser Ile Val Thr Glu Thr Lys Ser

95 100 105

50 aca aaa att ttg gta gac agc agc ctg act ctg aat atc ctg agt gtt 507

Thr Lys Ile Leu Val Asp Ser Ser Leu Thr Leu Asn Ile Leu Ser Val

110 115 120 125

55 tca ttt gct ttc atg ggc atc att atc atc tct gtc agc ctg gct ggt 555

Ser Phe Ala Phe Met Gly Ile Ile Ile Ile Ser Val Ser Leu Ala Gly

130 135 140

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Leu His Pro Ala Ser Glu Gln Cys Leu Gln Ser Lys Glu Leu Arg Pro

145 150 155

65 act gaa tat cat tac tac caa ttc ttg gac agg aac gag tgc ttt gcc 651

Thr Glu Tyr His Tyr Tyr Gln Phe Leu Asp Arg Asn Glu Cys Phe Ala

160 165 170

70 gcc aag tct gtt ctg gct gga gtc ttt tca ctg atg ctg atc agt act 699

Ala Lys Ser Val Leu Ala Gly Val Phe Ser Leu Met Leu Ile Ser Thr

175 180 185

atg ttg gaa ctt ggc ctg gct gtc ctc act gcc atg ctg tgg tgg aaa 747
 Met Leu Glu Leu Gly Leu Ala Val Leu Thr Ala Met Leu Trp Trp Lys
 190 195 200 205

5 cag agt cac tct aac atc cct ggg aat gtt atg ttc ctg cca cat agc 795
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 210 215 220

10 tca aat aat gac tcc aac atg gaa tca aag gta ctt tgt aac ccc tca 843
 Ser Asn Asn Asp Ser Asn Met Glu Ser Lys Val Leu Cys Asn Pro Ser
 225 230 235

15 tat gag gaa caa ttg gtt tgt taa gaaaaacaaa acaaaacaaa actaaatacc 897
 Tyr Glu Glu Gln Leu Val Cys
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5	Trp Gln Asp Ser Leu Lys Lys His Leu Lys Ala Glu Ile Lys Val Met	
	35	40 45
10	Ala Ala Ile Gln Ile Met Cys Ala Val Met Val Leu Ser Leu Gly Ile	
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15	Ile Leu Ala Ser Val Pro Ser Asn Leu His Phe Thr Ser Val Phe Ser	
	65	70 75 80
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	Val Ser Gly Ile Leu Ser Ile Val Thr Glu Thr Lys Ser Thr Lys Ile	
	100	105 110
25	Leu Val Asp Ser Ser Leu Thr Leu Asn Ile Leu Ser Val Ser Phe Ala	
	115	120 125
30	Phe Met Gly Ile Ile Ile Ile Ser Val Ser Leu Ala Gly Leu His Pro	
	130	135 140
35	Ala Ser Glu Gln Cys Leu Gln Ser Lys Glu Leu Arg Pro Thr Glu Tyr	
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40	His Tyr Tyr Gln Phe Leu Asp Arg Asn Glu Cys Phe Ala Ala Lys Ser	
	165	170 175
45	Val Leu Ala Gly Val Phe Ser Leu Met Leu Ile Ser Thr Met Leu Glu	
	180	185 190
	Leu Gly Leu Ala Val Leu Thr Ala Met Leu Trp Trp Lys Gln Ser His	
	195	200 205
50	Ser Asn Ile Pro Gly Asn Val Met Phe Leu Pro His Ser Ser Asn Asn	
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 Met Ile Pro Gln
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 35 gta gtg acc aat gag acc atc aca acg att tca cca aat gga atc aac 223
 Val Val Thr Asn Glu Thr Ile Thr Thr Ile Ser Pro Asn Gly Ile Asn
 5 10 15 20
 40 ttt ccc caa aaa gac gag tcc cag cct acc caa cag agg caa gac agc 271
 Phe Pro Gln Lys Asp Glu Ser Gln Pro Thr Gln Gln Arg Gln Asp Ser
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 45 ctg aag aaa cat cta aag gct gag atc aaa gtg ata gtg gca atc cag 319
 Leu Lys Lys His Leu Lys Ala Glu Ile Lys Val Ile Val Ala Ile Gln
 40 45 50
 50 atc atg tgt gct gtg aca gtg ttg gct ctg gga atc att ttg gca tct 367
 Ile Met Cys Ala Val Thr Val Leu Ala Leu Gly Ile Ile Leu Ala Ser
 55 60 65
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 Val Pro Pro Val Pro Tyr Phe Asn Ser Val Phe Ser Val Leu Leu Lys
 70 75 80
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 Ser Gly Tyr Pro Phe Ile Gly Ala Leu Phe Phe Ile Ala Ser Gly Ile
 85 90 95 100
 60 ttg tcc atc att acg gag aga aag tca aca aaa cct ttg gta gat gcc 511
 Leu Ser Ile Ile Thr Glu Arg Lys Ser Thr Lys Pro Leu Val Asp Ala

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5	agc cta act ctg aat atc ctg agt gtt tca ttt gct ttc gtg ggc atc Ser Leu Thr Leu Asn Ile Leu Ser Val Ser Phe Ala Phe Val Gly Ile 120 125 130	559		
10	att atc atc tct gtc agc ctg gct ggt ttg cat cct gcc tca gag caa Ile Ile Ile Ser Val Ser Leu Ala Gly Leu His Pro Ala Ser Glu Gln 135 140 145	607		
15	tgc aag cag agc aag gag ctt agt cta att gaa cat gac tac tac caa Cys Lys Gln Ser Lys Glu Leu Ser Leu Ile Glu His Asp Tyr Tyr Gln 150 155 160	655		
20	cct ttc tac aac tca gac agg agt gaa tgt gcc gtc acc aag tct att Pro Phe Tyr Asn Ser Asp Arg Ser Glu Cys Ala Val Thr Lys Ser Ile 165 170 175 180	703		
25	ctg act gga gcc ctt tca gtg atg ctg atc atc agt gtg ttg gag ctt Leu Thr Gly Ala Leu Ser Val Met Leu Ile Ile Ser Val Leu, Glu Leu 185 190 195	751		
30	ggg ctg gct ttg ctc tct gcc atg ctg tgg ttg aga gag ggt gtt ctg Gly Leu Ala Leu Leu Ser Ala Met Leu Trp Leu Arg Glu Gly Val Leu 200 205 210	799		
35	act tcc ctt aga atg tga ttttttctgt cttataactc caataatgaa Thr Ser Leu Arg Met 215	847		
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Ile Leu Ala Ser Val Pro Pro Val Pro Tyr Phe Asn Ser Val Phe Ser
 65 70 75 80

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Val Leu Leu Lys Ser Gly Tyr Pro Phe Ile Gly Ala Leu Phe Phe Ile
 85 90 95

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Ala Ser Gly Ile Leu Ser Ile Ile Thr Glu Arg Lys Ser Thr Lys Pro
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Leu Val Asp Ala Ser Leu Thr Leu Asn Ile Leu Ser Val Ser Phe Ala
 115 120 125

20

Phe Val Gly Ile Ile Ile Ile Ser Val Ser Leu Ala Gly Leu His Pro
 130 135 140

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Ala Ser Glu Gln Cys Lys Gln Ser Lys Glu Leu Ser Leu Ile Glu His
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Asp Tyr Tyr Gln Pro Phe Tyr Asn Ser Asp Arg Ser Glu Cys Ala Val
 165 170 175

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Thr Lys Ser Ile Leu Thr Gly Ala Leu Ser Val Met Leu Ile Ile Ser
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Met Ile Pro
1

15 caa gta gtg acc agt gag act gtc aca gtg att tca cca aat gga atc 166
Gln Val Val Thr Ser Glu Thr Val Thr Val Ile Ser Pro Asn Gly Ile
5 10 15

20 agc ttt ccc caa aca gac aaa ccc cag cct tcc cac cag agc caa gac 214
Ser Phe Pro Gln Thr Asp Lys Pro Gln Pro Ser His Gln Ser Gln Asp
20 25 30 35

25 agc ctg aag aaa cat cta aag gct gag atc aaa gtg atg gcg gca atc 262
Ser Leu Lys Lys His Leu Lys Ala Glu Ile Lys Val Met Ala Ala Ile
40 45 50

30 cag atc atg tgt gct gtg atg gtg ttg agt ctg gga atc att ttg gca 310
Gln Ile Met Cys Ala Val Met Val Leu Ser Leu Gly Ile Ile Leu Ala
55 60 65

35 tct gtt ccc tcc aat cta cac ttt acc tca gtg ttt tcc atc ctg tta 358
Ser Val Pro Ser Asn Leu His Phe Thr Ser Val Phe Ser Ile Leu Leu
70 75 80

40 gaa tct ggc tac cca ttt gta gga gct ttg ttt ttt gcc atc tct gga 406
Glu Ser Gly Tyr Pro Phe Val Gly Ala Leu Phe Phe Ala Ile Ser Gly
85 90 95

45 att ctg tct att gtc aca gag aaa aag atg act aag cct ttg gtt cac 454
Ile Leu Ser Ile Val Thr Glu Lys Lys Met Thr Lys Pro Leu Val His
100 105 110 115

50 agc agc cta gcc ctg agc atc ctg agt gtc ctc tct gct ctt aca ggc 502
Ser Ser Leu Ala Leu Ser Ile Leu Ser Val Leu Ser Ala Leu Thr Gly
120 125 130

55 atc gct att ctc tct gtc agt ttg gct gct tta gag cct gcc ttg cag 550
Ile Ala Ile Leu Ser Val Ser Leu Ala Ala Leu Glu Pro Ala Leu Gln
135 140 145

60 caa tgt aag ctg gct ttc aca caa cta gac aca acc caa gac gct tat 598
Gln Cys Lys Leu Ala Phe Thr Gln Leu Asp Thr Thr Gln Asp Ala Tyr
150 155 160

65 cat ttc ttt agc cct gag cca tta aac agc tgc ttc gtg gcc aaa gct 646
His Phe Phe Ser Pro Glu Pro Leu Asn Ser Cys Phe Val Ala Lys Ala
165 170 175

70 gct ctg act gga gtc ttt tca ctg atg cta atc agc agt gtg ttg gag 694
Ala Leu Thr Gly Val Phe Ser Leu Met Leu Ile Ser Ser Val Leu Glu
180 185 190 195

ctt ggc ctg gct gtc ctc act gcc aca ctg tgg tgg aaa cag agc tcc 742
 Leu Gly Leu Ala Val Leu Thr Ala Thr Leu Trp Trp Lys Gln Ser Ser
 200 205 210

5 tct gct ttc tct ggg aat gtg att ttc ctg tct cag aac tca aag aat 790
 Ser Ala Phe Ser Gly Asn Val Ile Phe Leu Ser Gln Asn Ser Lys Asn
 215 220 225

10 aaa tcc agt gta tct tca gag tca ctt tgt aac cct aca tat gaa aac 838
 Lys Ser Ser Val Ser Ser Glu Ser Leu Cys Asn Pro Thr Tyr Glu Asn
 230 235 240

15 ata ttg act tca taa gaattaagta gaggttatat agcagaaaaa tctgtcttta 893
 Ile Leu Thr Ser
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acatgattta gaaaagccat ttactgtgtg acaacaatgc ttaatatctt gatattcttaa 953

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 35 40 45

60 Ala Ala Ile Gln Ile Met Cys Ala Val Met Val Leu Ser Leu Gly Ile
 50 55 60

5 Ile Leu Ala Ser Val Pro Ser Asn Leu His Phe Thr Ser Val Phe Ser
 65 70 75 80

10 Ile Leu Leu Glu Ser Gly Tyr Pro Phe Val Gly Ala Leu Phe Phe Ala
 85 90 95

15 Ile Ser Gly Ile Leu Ser Ile Val Thr Glu Lys Lys Met Thr Lys Pro
 100 105 110

20 Leu Val His Ser Ser Leu Ala Leu Ser Ile Leu Ser Val Leu Ser Ala
 115 120 125

25 Leu Thr Gly Ile Ala Ile Leu Ser Val Ser Leu Ala Ala Leu Glu Pro
 130 135 140

30 Ala Leu Gln Gln Cys Lys Leu Ala Phe Thr Gln Leu Asp Thr Thr Gln
 145 150 155 160

35 Asp Ala Tyr His Phe Phe Ser Pro Glu Pro Leu Asn Ser Cys Phe Val
 165 170 175

40 Ala Lys Ala Ala Leu Thr Gly Val Phe Ser Leu Met Leu Ile Ser Ser
 180 185 190

45 Val Leu Glu Leu Gly Leu Ala Val Leu Thr Ala Thr Leu Trp Trp Lys
 195 200 205

50 Gln Ser Ser Ser Ala Phe Ser Gly Asn Val Ile Phe Leu Ser Gln Asn
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Met Arg Leu Gln
1

25 ctt ggc acc aag aac att ggg tgg gac tgc ttt cca aag gac atc att 164
Leu Gly Thr Lys Asn Ile Gly Trp Asp Cys Phe Pro Lys Asp Ile Ile
5 10 15 20

30 atc cac aaa aga gag aaa act gga cat aca tat gaa aaa gaa gat gac 212
Ile His Lys Arg Glu Lys Thr Gly His Thr Tyr Glu Lys Glu Asp Asp
25 30 35

ctg ctg att gga gtg cct agt gaa gcc aca ctt ctt gga acc atc cag 260
Leu Leu Ile Gly Val Pro Ser Glu Ala Thr Leu Leu Gly Thr Ile Gln
40 45 50

35 cta cta tgt gcc ctg ata ctt gca agc ttt ggg ggc att ttg gtg tca 308
Leu Leu Cys Ala Leu Ile Leu Ala Ser Phe Gly Gly Ile Leu Val Ser
55 60 65

40 gct tcc tac ttc aac cca gaa gtt tcc acc act ttg ata tct gga tac 356
Ala Ser Tyr Phe Asn Pro Glu Val Ser Thr Thr Leu Ile Ser Gly Tyr
70 75 80

45 ctg ttt att gga tcc ctc tgt ttt gcc att gct gga att ctc tcc att 404
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50 atc tct gaa aaa atc tca acc aaa ccc ttt gca ctg agc agc ctg gcc 452
Ile Ser Glu Lys Ile Ser Thr Lys Pro Phe Ala Leu Ser Ser Leu Ala
105 110 115

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Ser Asn Val Ala Ser Ser Val Val Ala Val Ile Gly Leu Phe Leu Phe
120 125 130

55 acc tat tgt ctg ata gcc ctg ggg agt gct ttc cca cac tgt aac tca 548
Thr Tyr Cys Leu Ile Ala Leu Gly Ser Ala Phe Pro His Cys Asn Ser
135 140 145

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Glu Lys Lys Phe Leu Ser Leu Leu Ser Tyr Leu Lys Ser His His Trp

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10	gct ctg ggg atg atg ctt ctc ttc act gtg ctg gag gtt ttc cta gct Ala Leu Gly Met Met Leu Leu Phe Thr Val Leu Glu Val Phe Leu Ala 185 190 195	692		
15	gga tac agt tct atc ttt tgg tgg aag caa gtc tac tcc aac aaa cct Gly Tyr Ser Ser Ile Phe Trp Trp Lys Gln Val Tyr Ser Asn Lys Pro 200 205 210	740		
	ggg ggt aca ttt ttc ttg cct caa tca caa gat cat acc caa ctt gtc Gly Gly Thr Phe Phe Leu Pro Gln Ser Gln Asp His Thr Gln Leu Val 215 220 225	788		
20	aaa agc aat ttg ttg caa taa tggatatgaa ataaaaaaga agaaagaaga Lys Ser Asn Leu Leu Gln 230	839		
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55	Gly Thr Ile Gln Leu Leu Cys Ala Leu Ile Leu Ala Ser Phe Gly Gly 50 55 60			
60	Ile Leu Val Ser Ala Ser Tyr Phe Asn Pro Glu Val Ser Thr Thr Leu 65 70 75 80			
	Ile Ser Gly Tyr Leu Phe Ile Gly Ser Leu Cys Phe Ala Ile Ala Gly			

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10	Ser Ser Leu Ala Ser Asn Val 115	Ala Ser Ser Val Val 120	Ala Val Ile Gly 125
15	Leu Phe Leu Phe Thr Tyr 130	Cys Leu Ile Ala Leu Gly 135	Ser Ala Phe Pro 140
20	His Cys Asn Ser Glu Lys 145	Lys Phe Leu Ser 150	Leu Leu Ser Tyr Leu Lys 155 160
25	Ser His His Trp Lys Asn Glu Asp Lys 165	Asn Cys Tyr Leu Ala Tyr Val 170 175	
30	Gly Ala Met Ser Ala Leu Gly Met Met Leu Leu Phe Thr Val Leu Glu 180 185 190		
35	Val Phe Leu Ala Gly Tyr Ser Ser Ile Phe Trp Trp Lys Gln Val Tyr 195 200 205		
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	Thr Val Ser Met Asn Thr Val Thr Ser Pro Gly Pro Met Ala Asn Ser	
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15	gta tat gtg gtg gca ccg ccc aac agt tat cct gtg gtc cca gga acc	146
	Val Tyr Val Val Ala Pro Pro Asn Ser Tyr Pro Val Val Pro Gly Thr	
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20	gtg cct cag atg cct att tat ccc agc aat cag cct caa gtc cat gtg	194
	Val Pro Gln Met Pro Ile Tyr Pro Ser Asn Gln Pro Gln Val His Val	
	50 55 60	
25	att tct ggt cat ctg cct ggt ttg gtg cca gct atg acc gaa cca cct	242
	Ile Ser Gly His Leu Pro Gly Leu Val Pro Ala Met Thr Glu Pro Pro	
	65 70 75	
	gcc cag aga gtc ttg aaa aag gga caa gtc cta ggg gcc atc cag atc	290
	Ala Gln Arg Val Leu Lys Lys Gly Gln Val Leu Gly Ala Ile Gln Ile	
30	80 85 90	
	ctg atc ggc ctg gtg cac atc ggc ctg ggc tcc atc atg atc acc aac	338
	Leu Ile Gly Leu Val His Ile Gly Leu Gly Ser Ile Met Ile Thr Asn	
	95 100 105	
35	ctc ttc agc cac tat aca cct gtc tcc ctc tat gga ggt ttc ccc ttc	386
	Leu Phe Ser His Tyr Thr Pro Val Ser Leu Tyr Gly Gly Phe Pro Phe	
	110 115 120 125	
40	tgg gga ggc atc tgg ttt atc att tca gga tct ctc tca gtg gcg gca	434
	Trp Gly Gly Ile Trp Phe Ile Ile Ser Gly Ser Leu Ser Val Ala Ala	
	130 135 140	
45	gaa act cag ccc aat tca cct tgc ctg ctg aat ggc agt gtg ggc ttg	482
	Glu Thr Gln Pro Asn Ser Pro Cys Leu Leu Asn Gly Ser Val Gly Leu	
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	aac atc ttc agc gcc atc tgt tct gcg gtg ggc atc atg ctc ttc atc	530
	Asn Ile Phe Ser Ala Ile Cys Ser Ala Val Gly Ile Met Leu Phe Ile	
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	Thr Asp Ile Ser Ile Ser Ser Gly Tyr Ile Tyr Pro Ser Tyr Tyr Pro	
	175 180 185	
55	tac cag gag aac ttg ggt gtg aga aca ggc gtg gct att tct agt gtg	626
	Tyr Gln Glu Asn Leu Gly Val Arg Thr Gly Val Ala Ile Ser Ser Val	
	190 195 200 205	
60	cta ctc atc ttc tgc ctc ttg gag ctc agc att gca agt gtg tcc tcc	674
	Leu Leu Ile Phe Cys Leu Leu Glu Leu Ser Ile Ala Ser Val Ser Ser	

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	His Phe Gly Cys Gln Val Ala Cys	Cys His Tyr Asn Asn Pro Gly Val		
	225	230	235	
10	gtc att cca aat gtc tat gca gca aac cca gtg gtc atc cca gaa cca	770		
	Val Ile Pro Asn Val Tyr Ala Ala Asn Pro Val Val Ile Pro Glu Pro			
	240	245	250	
15	cca aac cca ata cca agt tat tcc gaa gta gtt caa gac tcc aga taa	818		
	Pro Asn Pro Ile Pro Ser Tyr Ser Glu Val Val Gln Asp Ser Arg			
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	35 40 45			
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	50 55 60			
55	His Leu Pro Gly Leu Val Pro Ala Met Thr Glu Pro Pro Ala Gln Arg			
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	Val Leu Lys Lys Gly Gln Val Leu Gly Ala Ile Gln Ile Leu Ile Gly			
	85 90 95			
60	Leu Val His Ile Gly Leu Gly Ser Ile Met Ile Thr Asn Leu Phe Ser			
	100 105 110			

5 His Tyr Thr Pro Val Ser Leu Tyr Gly Gly Phe Pro Phe Trp Gly Gly
 115 120 125

10 Ile Trp Phe Ile Ile Ser Gly Ser Leu Ser Val Ala Ala Glu Thr Gln
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15 Pro Asn Ser Pro Cys Leu Leu Asn Gly Ser Val Gly Leu Asn Ile Phe
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20 Ser Ala Ile Cys Ser Ala Val Gly Ile Met Leu Phe Ile Thr Asp Ile
 165 170 175

25 Ser Ile Ser Ser Gly Tyr Ile Tyr Pro Ser Tyr Tyr Pro Tyr Gln Glu
 180 185 190

30 Asn Leu Gly Val Arg Thr Gly Val Ala Ile Ser Ser Val Leu Leu Ile
 195 200 205

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 210 215 220

40 Cys Gln Val Ala Cys Cys His Tyr Asn Asn Pro Gly Val Val Ile Pro
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Met Ala Gly Gln Ala Pro Thr Ala Val Pro Gly Ser
1 5 10

15 gtt act gga gaa gtc tca cga tgg cag aac cta gga cct gcc cag cct 158
Val Thr Gly Glu Val Ser Arg Trp Gln Asn Leu Gly Pro Ala Gln Pro
15 20 25

20 gca cag aaa gta gcc cag ccc caa aac ctg gtt cca gat ggg cac ctt 206
Ala Gln Lys Val Ala Gln Pro Gln Asn Leu Val Pro Asp Gly His Leu
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25 gag aaa gcc ctg gag gga agt gac ctt cta cag aag ttg ggt ggc ttt 254
Glu Lys Ala Leu Glu Gly Ser Asp Leu Leu Gln Lys Leu Gly Gly Phe
45 50 55 60

30 cac atc gcc att gct ttt gca cac ttg gcc ttt ggt ggc tac ttg atc 302
His Ile Ala Ile Ala Phe Ala His Leu Ala Phe Gly Gly Tyr Leu Ile
65 70 75

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Ser Thr Val Lys Asn Leu His Leu Val Val Leu Lys Cys Trp Tyr Pro
80 85 90

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Leu Trp Gly Thr Val Ser Phe Leu Val Ala Gly Met Ala Ala Met Thr
95 100 105

40 aca gtg aca ttc cct aag acc tct ctg aag gtt ctc tgt gtg ata gcc 446
Thr Val Thr Phe Pro Lys Thr Ser Leu Lys Val Leu Cys Val Ile Ala
110 115 120

45 aac gtt atc agc ttg ttc tgc gca ctg gcc ggc ttc ttt gtc att gcc 494
Asn Val Ile Ser Leu Phe Cys Ala Leu Ala Gly Phe Phe Val Ile Ala
125 130 135 140

50 aag gac ctc ttc ctg gag ggt cct ttt cca tgg cca atc tgg aga cca 542
Lys Asp Leu Phe Leu Glu Gly Pro Phe Pro Trp Pro Ile Trp Arg Pro
145 150 155

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Tyr Pro Glu Pro Thr Thr Tyr Ile Gln Arg Leu Glu Leu Thr Leu Phe
160 165 170

tgc ttc acc ttc ctg gag atc ttc ctg tgc ggg tcc aca gcc atc aca 638
Cys Phe Thr Phe Leu Glu Ile Phe Leu Ser Gly Ser Thr Ala Ile Thr
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 15 <302> Structure of the gene encoding the human B lymphocyte
 differentiation antigen CD20 (B1)
 <303> J Immunol
 20 <304> 142
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 <303> Proc Natl Acad Sci USA
 40 <304> 85
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<303> J Immunol

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Leu Trp Gly Gly Ile Met Tyr Ile Ile Ser Gly Ser Leu Leu Ala Ala
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Cys Glu Pro Ser Asn Ser Ser Glu Lys Asn Ser Pro Ser Thr Gln Tyr
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Published:

— with international search report

(88) Date of publication of the international search report:
13 March 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: IDENTIFICATION OF NOVEL MS4A GENE FAMILY MEMBERS EXPRESSED BY HEMATOPOIETIC CELLS

(57) Abstract: Isolated nucleic acids encoding MS4A polypeptides, isolated MS4A polypeptides, and uses thereof. The disclosed MS4A nucleic acids and polypeptides can be used to generate a mouse model of atopic disorders, for drug discovery screens, and for therapeutic treatment of atopic disorders or other MS4A-related conditions.



WO 02/062946 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/48437

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07K 14/00

US CL : 530/350

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,871,930 A (BANDMAN et al) 16 February 1999 (see entire document, SEQ ID NO2).	1, SEQ ID NO:1

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"

document member of the same patent family

Date of the actual completion of the international search

30 October 2002 (30.10.2002)

Date of mailing of the international search report

16 DEC 2002

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks

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Washington, D.C. 20231

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Authorized officer

Michael A. Belyavsky

Telephone No. 703/308-0196

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/48437

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1, SEQ ID NO:1

Remark on Protest

☐
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

PCT/US01/48437

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

1-38. Claim 1, drawn to an isolated MS4a polypeptide, or functional portion thereof, comprising a polypeptide encoded by the nucleotide sequence of one specific SEQ ID NOs :1-38.

39-76. Claim 2-7, drawn to an isolated nucleic acid molecule encoding a MS4A polypeptide, comprising the nucleotide sequence of one specific odd-numbered SEQ ID NOs: 1-37 chimeric gene, vector and a host cell.

77-115. Claim 8, drawn to a methods of detecting a nucleic acid molecule that encodes a MS4A polypeptide, the methods comprising hybridizing the nucleic acid molecule comprising the nucleotide sequence of one specific odd-numbered SEQ ID NOs: 1-37 to the biological sample.

116-154. Claim 9, drawn to an antibody that specifically recognizes a MS4A polypeptide comprising a polypeptide encoded by the nucleotide sequence of one specific SEQ ID NOs :1-38.

155. Claim 10, drawn to a method for producing an antibody that specifically recognizes a MS4A polypeptide.

156. Claim 11, drawn to a method for detecting a level of MS4A polypeptide.

157-195. Claim 12, drawn to a methods for identifying a substance that modulates MS4A function, the method comprising isolating a MS4A polypeptide comprising a polypeptide encoded by the nucleotide sequence of one specific SEQ ID NOs :1-38.

196-234. Claims 13-16, drawn to a method for modulating MS4A function in a subject, the method comprising preparing a pharmaceutical composition, comprising a substance identified according to a methods for identifying a substance that modulates MS4A function, the method comprising isolating a MS4A polypeptide comprising a polypeptide encoded by the nucleotide sequence of one specific SEQ ID NOs :1-38.

235. Claim 17 and 18 drawn to a method for identifying a candidate compound as a modulator of MS4A gene expression.

236. Claim 19, drawn to a method for modulating MS4A function in a subject, the method comprising preparing a gene therapy vector.

This International Searching Authority considers that the international application does not comply with the requirements of unity of the invention (Rules 13.1, 13.2 and 13.3) for the reasons indicated below:

The invention listed as a Group 1-236 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of Groups 1-38 is considered an isolated MS4A polypeptide, or functional portion thereof, comprising a polypeptide encoded by the nucleotide sequence of one specific SEQ ID NOs :1-38.

The special technical feature of Group 39-76 is considered an isolated nucleic acid molecule encoding a MS4A polypeptide, comprising the nucleotide sequence of one specific odd-numbered SEQ ID NOs: 1-37 chimeric gene, vector and a host cell.

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The special technical feature of Groups 77-115 is considered a methods of detecting a nucleic acid molecule that encodes a MS4A polypeptide, the methods comprising hybridizing the nucleic acid molecule comprising the nucleotide sequence of one specific odd-numbered SEQ ID NOs: 1-37 to the biological sample.

The special technical feature of Groups 116-154 is considered an antibody that specifically recognizes a MS4A polypeptide comprising a polypeptide encoded by the nucleotide sequence of one specific SEQ ID NOs :1-38.

The special technical feature of Group 155 is considered a method for producing an antibody that specifically recognizes a MS4A polypeptide.

The special technical feature of Group 156 is considered a method for detecting a level of MS4A polypeptide.

The special technical feature of Group 157-195 is considered a methods for identifying a substance that modulates MS4A function, the method comprising isolating a MS4A polypeptide comprising a polypeptide encoded by the nucleotide sequence of one specific SEQ ID NOs :1-38.

The special technical feature of Group 196-234 is considered a method for modulating MS4A function in a subject, the method comprising preparing a pharmaceutical composition, comprising a substance identified according to a methods for identifying a substance that modulates MS4A function, the method comprising isolating a MS4A polypeptide comprising a polypeptide encoded by the nucleotide sequence of one specific SEQ ID NOs :1-38.

The special technical feature of Group 235 is considered a method for identifying a candidate compound as a modulator of MS4A gene expression.

The special technical feature of Group 236 is considered a method for modulating MS4A function in a subject, the method comprising preparing a gene therapy vector.

Accordingly, Groups 1-236 are not so limited by the same or a corresponding special technical feature within meaning of PCT Rule 13.2 so as to form a single general inventive concept.

Continuation of B. FIELDS SEARCHED Item 3:

STN, WEST, MEDLINE, BIOSIS

search terms: SEQ ID NO:1, Tedder T; Taylor, J; Liang Y